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A STUDY OF THE HISTOLOGIC CHANGES INDUCED IN LEAVES BY CERTAIN LEAF-SPOTTING FUNGI[1]

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INTRODUCTION

Just why many necrogenic fungi parasitic upon the leaves of plants make only a limited growth resulting in definite lesions known as "spots" has not been satisfactorily explained. So far as the writer is aware there is recorded in literature no comprehensive investigation on the subject. In the many papers dealing with leaf-spotting fungi certain suscept reactions which may have some limiting influence upon the growth of the pathogene have occasionally been recorded. No doubt in many cases the reasons for such limitation of growth are purely physiological in character and have to do with the biochemical relation of pathogene and suscept. In a few cases there have been reported certain histologic changes in the suscept brought about as a result of the invasion of a necrogenic pathogene. Such histologic changes involve the revival of a meristematic condition in certain cells of the mesophyll resulting in the formation of new cells and an enlargement of these cells as well as of cells already in existence, with the ultimate production of a definite cicatrice separating the diseased portion from the healthy. Such a condition is reported as being more or less common in the leaves of higher plants when subjected to mechanical injury (Wylie, 21).

As a result of recently published work on the response of leaves to wounding and at the suggestion of Professor H. H. Whetzel, the present investigation was undertaken. The purpose of these studies has been to determine to what extent similar histologic changes are induced by leaf spotting fungi and whether such suscept responses play a major rôle in the restriction of their necrogenic activities.

[1] This paper presents a part of the author's doctorate investigations on the pathological histology of leaf lesions. Grateful acknowledgement is made of the suggestions and criticisms of Professors H. H. Whetzel and A. J. Eames, under whose direction the work has been done.

It was deemed desirable to study as many different leaf-spot diseases as possible in order to arrive at any conclusions of general application. It was also necessary in each case to study a large number of sections to be sure of the true nature of the changes which take place. These changes were found to vary greatly with the proximity of the spot to veins and islet borders. In all probability the age of the leaf at the time of infection and also environmental factors play an important part in the type of suscept reaction. In the case of wounding, the evidence of other workers goes to show that the moisture content of the atmosphere has a marked influence upon the type of healing tissue formed (Blackman and Matthaei, 1; Samuel, 17). It was also thought advisable to study, in a few cases at least, the response of the suscept to artificial wounding.

All of the material used in these investigations was collected in the vicinity of Ithaca, New York. Specimens of the diseased material, together with representative slides, have been deposited in the herbarium of the Department of Plant Pathology, Cornell University.

HISTORICAL REVIEW

While there are numerous references in literature to the healing of wounds in woody stems and the histologic changes resulting in callus formation, there has been published comparatively little regarding the reaction of leaves to mechanical injury and still less with respect to their reaction to invasion by necrogenic pathogenes.

Such references are scattered through various journals and in many cases are merely short paragraphs in a publication dealing primarily with the life history of the pathogene involved. This being the case, the author makes no claim to having exhaustively searched such literature but feels that the more important papers dealing with the subject have been consulted.

Bretfeld (2) states that in *Camellia japonica* wounds are healed simply by drying of the wound surface but that in *Bryophyllum sp.*, for example, there is often formed a large and peculiar periderm and that in general the arrangement of this periderm is parallel to the edge of the wound.

According to Pierce (14) the shot-hole lesion in leaves of almond caused by *Cercospora circumcissa* is bounded by partially dead and thickened tissues.

Frank (5, p. 63–67) states that wounds made with a scalpel in the leaves of *Leucojum vernum* are healed by callus formation, while insect wounds in the leaves of *Cornus sanguinea* are healed by the production of wound cork. In the former case the healing tissue consists of a mass of enlarged cells with suberized walls while in the latter case a definite periderm is formed.

Massart (11) describes and figures a cicatrice in a wounded leaf of *Nuphar sp.* formed about the lacunae. He says that the age of the tissue is important in determining the extent of cicatrice formation.

Miehe (13), studying wounded leaves of *Tradescantia sp.*, reports that the bared epidermal cells grow out and form a callus which at completion forms a cork layer.

Blackman and Matthaei (1) removed leaves of *Prunus laurocerasus* var. *rotundifolia* and placed the petioles in a beaker of water. When these leaves were wounded no healing tissue was formed. If, however, a sufficient number of cells were killed about the edge of the wound, an abscission layer was formed. The wounded portion eventually dropped out and the walls of the callus cells became cuticularized. Leaves wounded on the plants by cutting formed an abscission layer very quickly. The wounded portion did not fall out but division of the newly formed cells continued until a periderm of several layers of cork resulted.

Van Beusekom (18) noted that insect wounds on the tips of leaves of *Gnetum gnemon* brought about the formation of adventitious buds, and when the swollen tips were sectioned they showed multiplication and enlargement of the cells of the spongy parenchyma.

Wyneken (24) studied wound reactions in leaves in a large number of species of the higher plants and found that in some species a definite wound cork was formed. In such cases the outside layer of cells always had suberized walls, while in some cases lignification appeared in the walls of those cells lying next the suberized layer. In other species a callus composed of irregular, thick-walled cells was formed about the wound.

Higgins (7), working with *Coccomyces sp.* on species of *Prunus*, found that there was considerable variation in the deciduous character of the lesions in different species and even in the same species. He states that the separation is due to the abrupt enlargement of a layer of cells at some distance from the ends of the mycelium. The enlarged cells lose their chloroplasts and nuclei, and only a thin layer of protoplasm lines the walls.

A definite case of a barrier being laid down is given by Hesler (6), who worked with *Sphaeropsis malorum* on leaves of *Pyrus malus*. Where the lesion is not bounded by veins it is surrounded by a plate of cells which limits, for a time at least, the advance of the pathogene. The apparent stimulation results eventually in hyperplasia of the spongy tissue. The diseased cells gave a positive test for suberin.

Wylie (21), who severely wounded leaves of several species of higher plants, found that healing takes place quickly and the leaf continues to function. In a later paper (22), he discusses the structure of the healing tissues in species of widely different habitats. He found that this tissue is largely

formed by the development of new cells resulting from mitoses which establish walls parallel to the wounded edge of the leaf. All cell-layers of the blade share in this work, including the epidermis in case its cells are large. In a still later paper (23) he describes the healing process as embracing first the formation of a pseudo-cicatrice as a result of the death and collapse of exposed cells along the wounded edge, which is followed by the formation of the cicatrice proper.

Woit (20) studied wounded leaves in a number of species belonging to different families of the higher plants and found that many cells in full grown leaves are stimulated to division and new growth. Cells of the collenchyma, conductive tissue, and hairs are often active in the wound reaction. In a moist atmosphere callus is formed, while a freely exposed wound usually develops wound cork only.

In a study of *Prunus amygdalus*, attacked by *Clasterosporium carpophilum*, *Samuel* (17) found that the infected tissues in young leaves are invariably abscissed. In the older leaves an abscission layer is formed but the infected tissues do not always fall out. The abscission layer begins with the swelling of cells in a narrow zone at some distance from the margin of the invaded area. Both palisade and spongy parenchyma cells are involved and complete occlusion of the intercellular spaces results. Where the invaded portion is abscissed, suberization of the walls of the cells along the abscis line occurs soon after the cuticle ruptures. Subsequent divisions of the meristematic layer result in the formation of a few layers of brick-shaped cells which become suberized and slightly lignified. Where abscission does not occur the initial changes are similar, but in the later stages there is lignification of the walls of the cells on the inner side of the occluded zone and a suberization of the walls of the meristematically formed cells.

McWhorter (12, p. 19–21) describes the formation of a meristem in the leaves of *Carica papaya* infected with *Cladosporium papayae*. This meristem acts like a cork cambium, cutting off cork cells. The fungus is able to penetrate these cork cells to a limited extent.

It is evident from these records that mechanically wounded leaves of many plants respond by histologic modification of adjacent tissues. They also indicate that, at least in certain fungous diseases, similar healing-out modifications take place in the tissues about the lesion. However, one would be led to conclude from the very limited number of such cases recorded that such a response to the activity of pathogenes is relatively rare.

METHODS

As the material was collected small segments were removed from the diseased leaves by means of a razor blade. These segments included both

diseased and healthy tissue. When the lesions were small the portion removed from the leaf included the whole of the necrotic area. In the case of the larger lesions this procedure was not possible, as the size of the segment was necessarily limited by the difficulties of fixation. As far as possible only mature leaves with well developed lesions were used in these studies, several segments being taken from each of a number of infected leaves.

Immediately the segments were removed from the leaf they were placed in shell vials containing medium chromo-acetic fixing solution (3). In the case of some material, a vacuum-pump was used to insure rapid removal of air from the tissues. Other material was merely forced under the liquid by means of a wad of cheesecloth placed on top of the segments in the vial. The killing was perfectly satisfactory by both methods. The material remained in the fixing solution for from 24 to 36 hours and on removal was washed in running water for an equal length of time, after which it was imbedded in paraffin (53°–55° F.) in the usual manner.

Whenever time would permit, freehand sections were also made of the fresh material. At this time studies were made on the extent of the mycelial development, and microchemical tests were applied to determine the nature of the changes in the walls and contents of those cells lying at or near the edge of the lesion. In the study of freehand sections of freshly collected material cotton blue was used as a mycelial stain. This proved satisfactory in some leaves but was not so effective with others owing to the heavy staining of the chloroplasts. Tests for lignified tissue were made with phloroglucin and hydrochloric acid. The presence of cutin and suberin was determined by staining with Sudan III. This was done by boiling the sections in a drop of stain on the slide, washing in water, and mounting in glycerin for examination. The ferric-chloride test was used to determine the presence of tannins.

These tests were repeated with sections made from material imbedded in paraffin. In testing for suberin it was not possible to boil the stain on the slide after the sections had been attached with albumen fixative. Good results were obtained by placing the slides in a Coplin jar containing the stain and keeping this at a temperature of 40° C. for 36 hours. The slides were then thoroughly rinsed in water and mounted in glycerin for examination.

In addition to cotton blue as a mycelial stain, both Durand's (4) and Vaughan's (19) staining methods were used. None of the methods mentioned proved entirely satisfactory in all cases. For the purpose of general histologic studies, sections were stained on the slide in Delafield's haemotoxylin; safranin; Delafield's haemotoxylin and safranin combined; and in

crystal violet combined with erythrosin (8). The latter combination gave a beautiful contrast and was particularly valuable in detecting lignified tissue. In many cases it also proved valuable in staining the mycelium.

Some of the plates were prepared with the aid of a camera lucida, this being used merely to get the proportions and the bare outline of cells. The remainder of the plates were prepared by inking in the cell walls on an enlargement made from a photomicrograph. The enlargement was then treated with a solution of potassium cyanide, followed by a solution of iodine-potassium iodide, after which it was thoroughly washed and dried. This treatment removed the image and resulted in a plain black and white drawing. With both methods the details were filled in freehand. In all cases the details shown in the plates represent the author's interpretation of the condition existing in the cells as gathered from the study of a large number of sections stained in various ways.

Where artificial wounding was practiced the wounds were made on mature leaves by means of a common leather punch making a circular hole 2 mm. in diameter. In this way a clean-cut wound was obtained. As far as possible, care was taken to avoid cutting into or near the larger veins. Several wounds were made in a single leaf, the number depending somewhat upon the size of the leaf. The wounded leaves were allowed to remain on the plant for a period of from 18 to 21 days. At the end of that period they were collected and segments removed, treated, and studied in the same manner as those from diseased leaves.

PRESENTATION OF DATA

The results of the present investigation are presented under two headings based on the type of histologic changes which result from infection by the various pathogenes on the suscepts dealt with in this paper.

In practically every case the leaf-spot lesion consists of a clearly defined central dead area surrounded by a zone of varying width and coloration in which the cells are still alive but diseased. Following the terminology used by Whetzel in his mimeographed text on symptomatology, this central region will be designated the "holonecrotic" region or area, while the marginal zone will be referred to as the "plesionecrotic" zone or region.

Unless otherwise stated, the mesophyll cells are of the usual thin-walled type containing nuclei and chloroplasts in a peripheral layer of cytoplasm. The epidermal cells also have a thin layer of cytoplasm about the walls, and nuclei are present.

Diseases in which a Definite Cicatrice is formed about the Margin of the Lesion

Three species of *Coccomyces* and one species of *Mycosphaerella*, all of which are parasitic on members of the family Rosaceae, together with one species of *Cercospora* parasitic on *Beta vulgaris*, were found to cause histologic changes of the type under discussion.

The term "cicatrice" as used in this paper is applied to a band of healing tissue about the lesion or wound, the cells of which have been derived by division and enlargement of the mesophyll cells. The epidermal cells are also frequently involved. This cicatrice ordinarily consists of two parts, the outer part which is a typical wound periderm, and the inner part which lies between the wound periderm and the normal tissue. This latter part is composed of somewhat modified cells evidently not derived from the activities of the wound phellogen.

Lesions produced by Coccomyces prunophorae Higgins on Prunus domestica L. In the healthy portions of the leaf of *Prunus domestica* the upper epidermal cells are large and somewhat irregular in outline. Both the inner and outer walls are convex but this is more marked in the case of the inner walls. The outer walls are noticeably thickened as are also the radial ones, while the inner are comparatively thin. Except that the cells are somewhat smaller, the structure of the lower epidermis is very similar to that of the upper. The palisade parenchyma is well-defined and consists of two or even three layers of elongated narrow cells. In a cross-section of the leaf the upper layer appears unbroken by intercellular spaces. The lower layer, however, is less compact, and intercellular spaces are evident between many of the cells. The cells of the spongy parenchyma are more or less irregular in size and shape and are rather loosely arranged. In the immediate vicinity of the larger veins the palisade parenchyma is replaced by enlarged, circular to irregularly shaped, thick-walled parenchyma cells, while the region of the spongy parenchyma is occupied by the principal bundle tissues with strengthening cells below. Where smaller veins are present the palisade parenchyma is still intact, but that portion of the spongy parenchyma not occupied by the bundle is filled with somewhat small, thick-walled parenchyma cells.

The necrotic lesions caused by the fungus vary considerably in size and appearance depending upon the age of the lesion and the extent of the infection. The smaller lesions are more or less circular in outline, the dark-brown holonecrotic area being surrounded by a narrow yellowish-green plesionecrotic zone. Where the lesions are numerous they frequently coalesce to form large, irregular areas. In the older spots the diseased portion

may drop completely out, leaving a clean-cut margin. This margin may or may not be thicker than the normal leaf.

A cross-section made through one of these lesions reveals the presence of a very definite cicatrice at the margin (Fig. 1). Throughout the whole

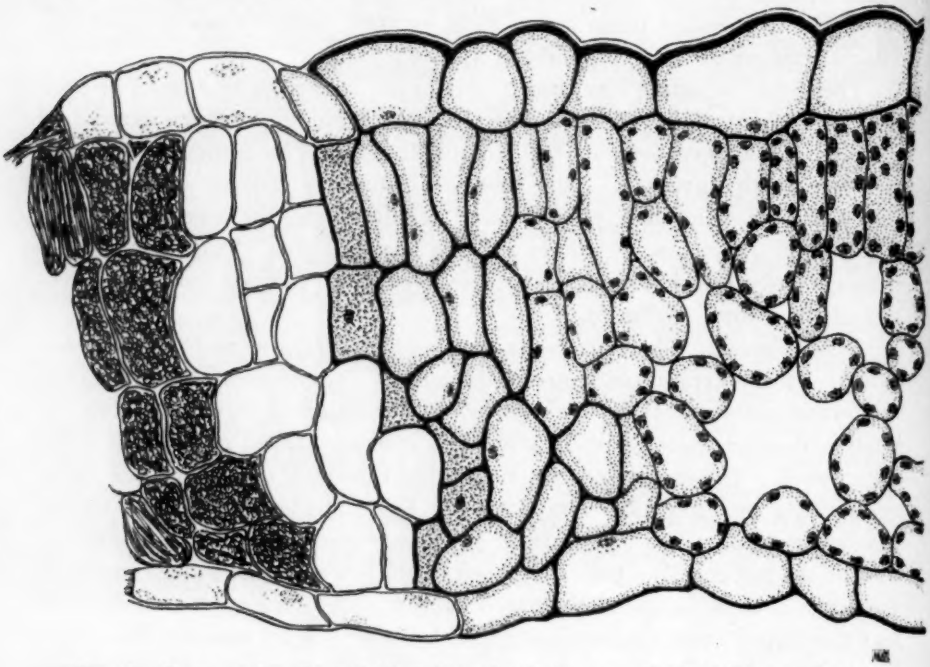

FIG. 1. Cross section through the edge of a lesion on a leaf of *Prunus domestica* caused by *Coccomyces prunophorae*. ×428.

region occupied by the cicatrice there has been division and multiplication of the cells of both the palisade and spongy parenchyma which has resulted in a band of tissue without intercellular spaces. The wound periderm is several cells in width. The phellem is made up of a mass of large cells whose thick walls are not only suberized but lignified as well. The cells lying nearest the lesion are filled with a dense granular substance resembling tannin, while the remaining cells are apparently devoid of contents. The epidermal cells of this layer also have suberized walls. Both a phellogen and phelloderm are present in the wound periderm. The cells comprising these two layers are thick-walled but this thickening is entirely cellulose in nature. Chloroplasts are absent from these cells. The phellogen consists of a single layer of cells which are filled with dense protoplasm, while the cells of the phelloderm contain only a peripheral layer. Nuclei are plainly visible in the cells of both of these layers. The inner part of the cicatrice is com-

posed of somewhat enlarged, thin-walled living cells, with only a few chloroplasts present.

All efforts failed to demonstrate the presence of the pathogene beyond the edge of the cicatrice.

The reaction to the leaf to wounding (Fig. 2) is not unlike that induced

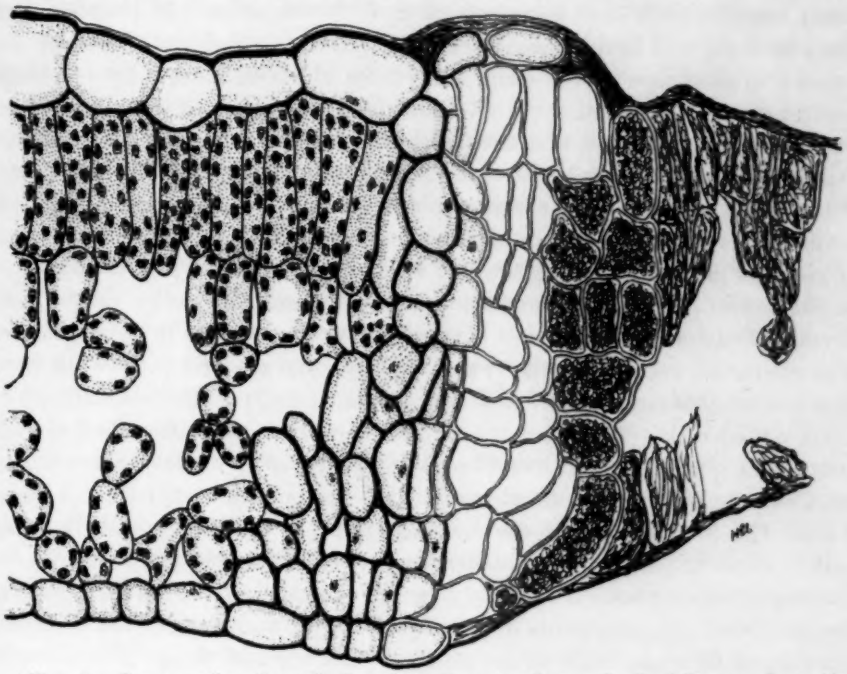

FIG. 2. Cross section through the edge of a wound on a leaf of *Prunus domestica* showing the cicatrice formed under these conditions. ×428.

by the pathogene in question. There are, however, certain differences which seem to be characteristic. The principal difference appears in the cells of the phellem layer. In the case of wounding, the cells are somewhat smaller and the radial arrangement is more definite. The leaf in the region of the cicatrice is usually somewhat thicker than normal. The cicatrice is formed at some little distance from the edge of the wound. At the edge of the wound the cells have entirely collapsed and this condition becomes less evident as we approach the cicatrice. Many of these collapsed cells, both in the palisade and in the spongy parenchyma, are more or less filled with the tannin-like substance already referred to.

Lesions produced by Coccomyces lutescens Higgins on Prunus virginiana L. The structure of the healthy leaf of *Prunus virginiana* is very similar to that of *Prunus domestica*. The leaf of the former species, however,

is somewhat thinner, and at intervals resin cavities are formed in the upper part of the palisade parenchyma.

The lesions on this suscept are also very similar in appearance to those on *Prunus domestica* and need no further description.

When viewed in cross-section the type of cicatrice is seen to differ in some respects from that described on *Prunus domestica*. At the edge next the lesion the leaf is fully one-third thicker than normal and gradually decreases in thickness to the point where the cicatrice merges into healthy leaf tissue. Throughout the whole cicatrice there has been a multiplication and division of the cells of the spongy parenchyma and also of the lower palisade cells which has resulted in the occlusion of the intercellular spaces. The wound periderm is of considerable width. The phellem layer, which is several cells wide, is made up of much enlarged cells, more or less irregular in shape, whose walls are lignified as well as suberized. While they are not so regular in their arrangement as are the phellem cells in the wound periderm in *Prunus domestica*, they evidently have their origin in the phellogen. The epidermal cells in this layer are slightly enlarged and their walls have also become suberized. The remainder of the wound periderm is made up of thick-walled cells. The outer one or two rows lying next the phellem constitute the phellogen and are filled with protoplasm while the remaining rows contain only a peripheral layer. No chloroplasts are present in these cells. The inner layers of the cicatrice is made up of thin-walled living cells. Those nearest the wound periderm contain no chloroplasts while the number of chloroplasts is relatively few in the cells adjacent to the normal tissue. Dead cells, remnants of the diseased area, may or may not be found clinging to the outer edge of the phellem. No trace of the pathogene could be found beyond the cicatrice.

The cicatrice which is formed as a result of wounding is so similar to that formed on *Prunus domestica* under similar conditions that little further description is necessary. The chief difference lies in the fact that it is not so extensive, there being at most only two layers of cells in the phellem and these very much enlarged.

Lesions produced by Coccomyces hiemalis Higgins on Prunus cerasus L. With certain minor differences the general structure of the leaf of *Prunus cerasus*, as studied in cross section, is similar to that of the *Prunus sp.* already described. The cells of both the lower and upper epidermis are irregular in size and shape. While some of the upper epidermal cells contain the usual thin layer of protoplasm, many of them are filled with a dense substance which stains deeply with haemotoxylin. An occasional cell of the lower epidermis is also filled with this same substance.

The type of lesion found on this suscept is very similar to that described on *Prunus domestica*. A study of a cross-section made through one of these

lesions shows a cicatrice formed at the margin which is almost identical with that described on *Prunus domestica*. Here we find the same phellem layer of enlarged cells having suberized and lignified walls and apparently lacking in cell contents. Between this layer and the normal tissue there is a layer of thick-walled living cells.

Wounding of the leaf of this suscept results in the formation of a cicatrice so similar in its general characters to that formed on *Prunus domestica* under the same conditions that no detailed description need be given.

Lesions produced by Coccomyces hiemalis Higgins on Prunus avium L. The structure of the healthy leaf of this suscept is essentially the same as that of *Prunus cerasus* and the type of lesion produced by the pathogene is the same as that produced by the other species of *Coccomyces* on the suscepts studied.

As in the case of the other species of *Prunus* dealt with in this paper, there is a definite cicatrice formed at the edge of the lesion. A study of a cross-section through this formation shows that in its general structure it is similar to that already described on *Prunus domestica*.

Wounded leaves formed a cicatrice in all respects like that formed on the other species of *Prunus* when wounding was practiced.

Lesions produced by Mycosphaerella sentina (Fr.) Schr. on Pyrus communis L. In the healthy leaf of *Pyrus communis* the lower epidermis is very much like the upper in its general characters. The outer cell walls of the latter, however, are somewhat thicker than are the outer cell walls of the former. In both cases occasional cells are partly filled with a dense staining material. The palisade parenchyma consists of two layers of closely packed cells, those of the upper layer being much longer than those of the lower. The cells of the spongy parenchyma are variable as to size and shape and are loosely arranged.

The lesions on the leaf are oval to irregular in outline, are entirely holonecrotic in character, and are brown in color with a grayish-white center. The margin is somewhat darker in color than is the main body of the lesion; is slightly raised and sharply defined.

At the edge of the holonecrotic area there is a very definite cicatrice laid down. When viewed in cross-section this is seen to be similar to that described on *Prunus domestica*. The outer edge of the cicatrice ends abruptly in normal tissue, and by none of the methods used was it possible to find any evidence of the pathogene beyond this barrier.

Cross-sections of leaves artificially wounded reveal the formation of a cicatrice at some little distance from the edge of the wound. The intervening cells are dead and in some cases the epidermis has apparently folded over the edge of the wound. In all respects this cicatrice is similar to that formed at the edge of the lesion caused by the pathogene.

Lesions produced by Cercospora beticola Sacc. on Beta vulgaris L. The healthy leaf of *Beta vulgaris* as seen in cross-section is rather open in structure. The upper epidermis is composed of cells which are more or less irregular in outline. The outer and radial walls are comparatively straight but the inner walls tend to curve inward, at times even forming an obtuse angle. The outer walls are considerably thickened, the radial walls somewhat less so, while the inner walls are only slightly thickened. The cells of the lower epidermis are somewhat different from those of the upper, being slightly longer, narrower, and with the inner wall more parallel to the outer. Both the outer and radial walls are noticeably thickened. The palisade parenchyma is very open in structure. Large intercellular spaces are present, and in cross section of the leaf the cells appear in single rows, or at most in groups of two or three, each row comprising two or three elongated cells placed end to end. In the material sectioned no large veins were present, but in the presence of small veins the structure of the palisade parenchyma was unaltered. The spongy parenchyma is also very open in structure. While the cells are a bit more irregular in shape and arrangement, this tissue does not differ essentially from the palisade parenchyma.

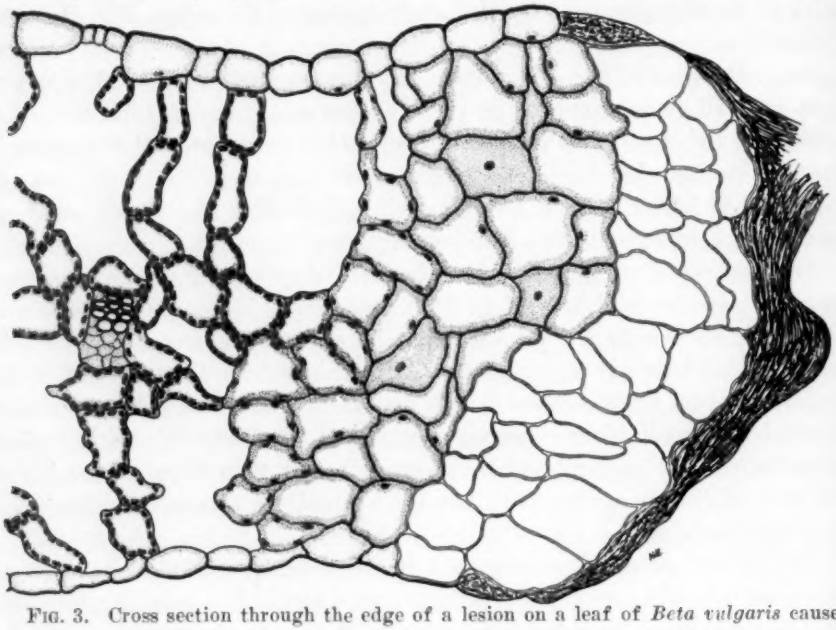

FIG. 3. Cross section through the edge of a lesion on a leaf of *Beta vulgaris* caused by *Cercospora beticola*. ×225.

The infected areas on the leaves appear as spots of varying size, more or less regular in outline, holonecrotic in character, the center of which is sunken and grayish-white in color, surrounded by a purplish-red margin.

A cross-section of the leaf made through one of these lesions (Fig. 3) shows that there has been a marked change in the character of the tissue adjacent to the sunken area. For some distance back from this region there has evidently been renewed meristematic activity in the cells of the mesophyll which has resulted in complete occlusion of the intercellular spaces and the formation of a definite cicatrice. In general the region occupied by the cicatrice is somewhat thinner than that of the healthy portion of the leaf, although there was considerable variation in this respect in the material examined. The variation may possibly have been due

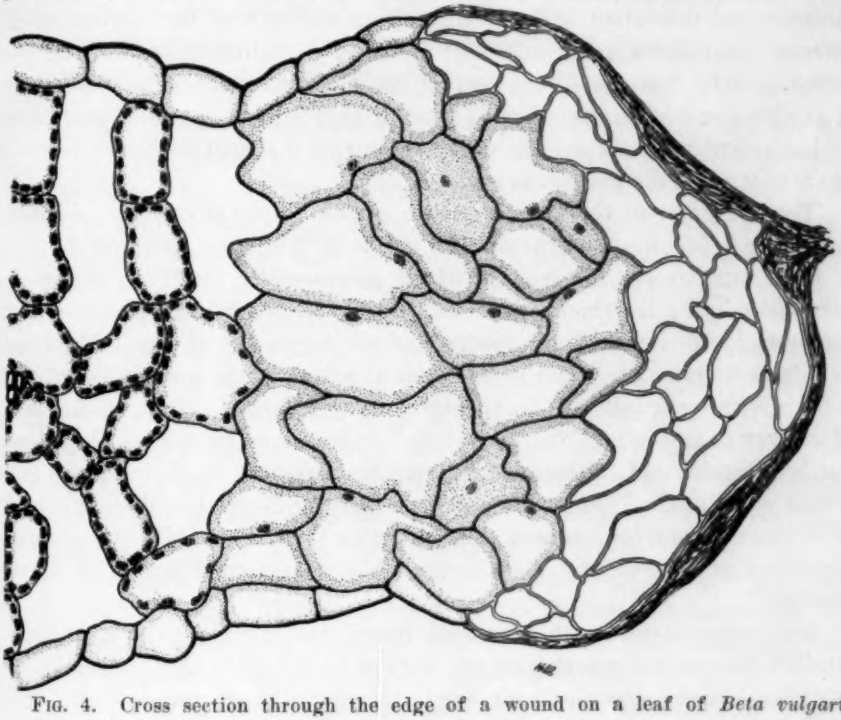

FIG. 4. Cross section through the edge of a wound on a leaf of *Beta vulgaris* showing the cicatrice formed under these conditions. ×300.

to differences in the maturity of the wounded leaves. The cicatrice does not differ essentially in character from those already described on species of *Prunus*. The cells, however, are larger and more irregular in shape, while the thickening of the walls is not so pronounced. There is a mass of dead collapsed cells attached to the outer edge of the phellem layer. All efforts failed to demonstrate the presence of the fungus either within or behind the cicatrice.

The cicatrice formed as a result of wounding (Fig. 4) is very similar in its general characters to that just described. The cells of the phelloderm

as shown in the illustration are very large but there is such a variation in this respect in the different sections that one cannot consider this a constant character of wounded leaves.

Discussion

The evidence presented in the foregoing pages shows that the species of *Prunus, Pyrus,* and *Beta* studied may be expected to react, when attacked by necrogenic pathogenes, by the formation of a cicatrice which isolates the infected portion from the healthy tissue. The character of this cicatrice and the extent of the healing tissue differs with the relation of the infected portion to veins and islet borders. Undoubtedly there is also variation with the age of the leaf at the time at which infection occurs. It is also possible, in fact quite probable, that environmental factors play an important part not only in the character of the cicatrice formed but in the initiation of the process as well.

The character of the cicatrice, with certain minor differences, is essentially the same in all of the species studied. The most striking features of this structure are the renewal of the meristematic condition in the mature mesophyll cells which results in a multiplication of cells in this region, the occlusion of intercellular spaces, and the formation of a definite wound periderm with its phellogen layer which is active in the production of cork cells. These cork cells are frequently greatly enlarged. This enlargement of a layer of cells about the "shot-hole" in leaves of *Prunus sp.* is pointed out by Higgins (7), although he makes no reference to their origin from a cork cambium. That such a cambium may be formed in diseased leaves of *Prunus amygdalus* has been clearly shown by Samuel (17), and a similar condition is reported by McWhorter (12) on diseased leaves of *Carica papaya.*

The experiments with wounded leaves indicate that in the species studied the natural reaction of the suscept to injury is the formation of a cicatrice. Such a cicatrice is similar in its general characters to that formed at the edge of necrotic lesions caused by pathogenic organisms. That the formation of a cicatrice of some nature, as a result of mechanical injury, is of common occurrence in the leaves of many higher plants is evident from the work of Blackman and Matthaei (1), Wyneken (24), Wylie (23), Woit (20), and others.

In the present work no attempt was made to study the development of the healing tissue nor the factors influencing its formation. The work of Samuel (17) is interesting in this connection and deals in considerable detail with the development of this healing tissue in *Prunus amygdalus,* while the work of Blackman and Matthaei (1) is of equal interest in connection

with the formation of healing tissues in leaves subjected to traumatic stimulation.

The fact that in no case where a typical wound periderm is formed about the edge of the lesion was it possible to find any evidence of the hyphae beyond this point would indicate that this periderm was an effectual barrier against the advance of the fungus. This is not conclusive proof, however, as certain physiological factors may be involved and, furthermore, McWhorter (12) has shown that this layer may be penetrated to a slight extent by *Cladosporium papayae*.

Diseases in which no Definite Cicatrice is Formed about the Edge of the Lesion

By far the larger number of the diseases studied were found to fall in this group. The species of pathogenes involved belong to several genera and occur upon a wide variety of suscepts among the flowering plants.

As there seems to be no correlation either between the species of suscept or the species of pathogene and the reaction of the suscept to the advance of the organism, the observations have been grouped according to the genus to which the pathogene belongs.

Lesions produced by Septoria petroselini Desm. on Apium graveolens L. Both the upper and the lower epidermis of the leaf of *Apium graveolens* are of medium thickness and are made up of rather large cells. The palisade parenchyma consists of a single row of rather short broad cells somewhat openly arranged. The spongy parenchyma is made up of cells which vary greatly in both shape and size, some being comparatively small and more or less globose in outline, while others are large and elongated.

The lesions caused by the fungus are small but often coalesce to form large, irregular areas. The main body of the lesion is holonecrotic in character and brownish-gray in color. This holonecrotic area is surrounded by a yellowish-green plesionecrotic zone which is variable in width.

A study of a cross-section through one of the older lesions (Fig. 5) reveals the fact that the cell contents have entirely disappeared and the walls have collapsed. The cell walls are brown, and there is an abundance of intercellular mycelium. The fungous hyphae are plainly visible in the plesionecrotic area and extend to the margin of normal leaf tissue. Those cells in the region of the tips of the advancing hyphae are apparently unchanged but in the intervening cells the contents are broken down and this condition is more complete nearer the necrotic area. In no case is there any evidence of cicatrice formation.

Lesions produced by Septoria podophyllina Pk. on Podophyllum peltatum L. In the healthy leaf of *Podophyllum peltatum* both the upper and

lower epidermis are composed of rather large, irregular, thin-walled cells. There is no well-defined palisade parenchyma but there is a layer of irregularly shaped, somewhat loosely arranged cells, lying just under the upper epidermis. The spongy parenchyma is open, particularly in the lower portion where the globose to elongate cells are arranged in chains, or groups, to enclose large air spaces.

On the material collected the lesions vary greatly in size and are more or less irregular to angular in outline. In many cases this irregularity in outline is due to veins at the edge of the lesion. The lesion is composed of a brown holonecrotic area surrounded by a yellowish-green plesionecrotic zone of varying width. This may be sharply delimited from the normal green tissue of the leaf or may blend gradually into it.

In the necrotic area the cell contents have disappeared and the walls have collapsed. The intercellular hyphae are abundant in this region.

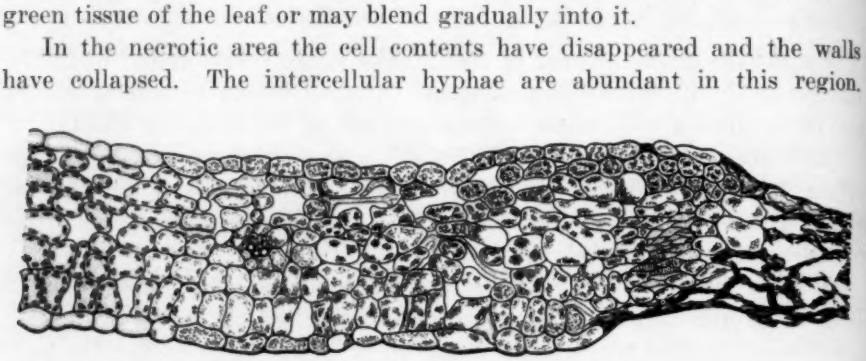

FIG. 5. Cross section through a diseased leaf of *Apium graveolens* showing the histologic changes induced by *Septoria petroselini*. ×171.

The hyphae extend through the plesionecrotic zone and at the outside limit of growth apparently lie between normal cells.

Artificial wounds were made in leaves of *Podophyllum peltatum*. There is no formation of a wound periderm such as is found in *Prunus sp.* but there are certain changes in the character of the contents and in the walls of those cells adjoining the wound. For some distance back from the point of wounding all of the leaf tissues are dead and collapsed, and these collapsed cells contain a brownish substance resembling tannin. Adjoining the inner edge of this collapsed area, and lying between it and normal tissue, there are a few cells which are filled with this brownish substance and whose walls give a positive reaction for suberin. In some cases these cells also may be partly collapsed. There is apparently no increase in either number or size of cells in this region.

Lesions produced by Septoria conspicua Ell. et Mart. on Steironema ciliatum (L.) Raf. The structure of the healthy leaf of *Steironema ciliatum* is rather open. The leaf is very thin but the upper epidermis and also the

lower are quite thick as compared with the total thickness of the leaf. Both epidermal layers are made up of large cells. The palisade parenchyma consists of a single layer of short, broad cells rather loosely arranged. The cells of the spongy parenchyma vary considerably both in size and shape.

On the leaves of this suscept the lesions are circular in outline and rather uniform in size. The central portion of the holonecrotic area is dark brown in color while the margin is a very dark brown. This area is surrounded by a pale yellowish plesionecrotic zone.

The diseased portion is very little thinner than is the normal leaf. In the holonecrotic area the epidermal cells have only partially collapsed. Many of the palisade cells in this region, as well as some of the cells of the spongy parenchyma, are completely filled with a dense granular substance which did not react to any of the stains used. These cells are only slightly shrunken, and in many parts of this area the intercellular spaces are completely filled with the large hyphae. In the plesionecrotic zone the cells of the mesophyll are filled with the same dense granular substance, and occasional intercellular strands of hyphae can be found. Beyond these granulated cells the tissue is normal. There is no other apparent change in the character of the cells in this region.

Lesions produced by Septoria cirsii Niessl. on Cirsium arvense (L.) Scop. The leaf of *Cirsium arvense* is open in structure. The epidermal cells are large and comparatively thick-walled. There is no well-defined palisade parenchyma but there is a layer of loosely arranged, irregular shaped cells lying just beneath the upper epidermis. The remainder of the mesophyll tissue is quite open, the cells varying greatly in both size and shape and arranged more or less in rows, or groups, enclosing large air spaces.

The lesions on the leaves are somewhat irregular in size and shape. The main portion of the lesion is holonecrotic in character, brownish in color, with a well-defined margin. This is surrounded by a narrow, greenish-yellow, plesionecrotic zone. These lesions frequently coalesce to form large irregular patches.

In the holonecrotic area the cell contents have completely disappeared, the cells have collapsed, and the leaf is much thinner at this point. Threads of hyphae are visible between these cells and also in the intercellular spaces of the plesionecrotic zone. The cells comprising this zone have their contents largely disorganized and the walls are partially collapsed. In no case was there any evidence of cicatrice formation.

Lesions produced by Septoria verbasicola B. et C. on Verbascum blattaria L. The healthy leaf of *Verbascum blattaria* is rather thick and in cross-section the cells of the upper epidermis are seen to be much larger

than those of the lower. In both cases the inner and outer walls are thickened. The palisade parenchyma is made up of two or three layers of rather short, broad cells. Occasionally several of the cells of the upper layer have broken down to form a large cavity. The cells of the spongy parenchyma are somewhat irregular in shape and rather compactly arranged.

The lesions in this suscept are variable in size, more or less oval in outline, and frequently coalesce. The holonecrotic center is grayish-white in color while the plesionecrotic area surrounding it is purplish-red and tends to blend into the normal green of the leaf.

In the holonecrotic area the cell contents have entirely disappeared and the walls are completely collapsed. The hyphae are abundant between these collapsed cells, can be seen in the intercellular spaces in the plesionecrotic area, and extend to the edge of normal tissue. The nuclei and chloroplasts have disappeared from most of the cells in this area, some being entirely empty although they still retain their shape. There is no multiplication of cells nor change in the character of the cell walls about the margin of the lesion.

Lesions produced by Septoria acerina Pk. on Acer pennsylvanicum L. The healthy leaf of *Acer pennsylvanicum* is comparatively thin and many of the cells of both the upper and lower epidermis are filled with a dense tannin-like substance. The palisade parenchyma is made up of a single layer of much elongated cells which are closely packed together. The spongy parenchyma is rather open in structure and is composed of cells which vary greatly in size and shape.

The lesions caused by this fungus are small, irregular in outline, and frequently coalesce to form larger areas. The holonecrotic center is dark brown in color and is surrounded by a broad, light-colored, clearly defined plesionecrotic zone.

With an occasional exception, the contents have entirely disappeared from the cells in the necrotic area. The epidermal cells and the cells of the spongy parenchyma have collapsed. Many of the palisade cells have also collapsed and those that have not are very much shortened. The intercellular hyphae are abundant throughout the whole of this area and extend through the plesionecrotic zone to the edge of normal tissue. The contents of the cells in this region are somewhat disorganized but apparently no shrinkage has taken place. Occasional palisade cells, or even groups of two or three of these cells, have densely granular contents. In no case was there any indication of a change in the character of the cell walls except that common to the necrotic area.

Lesions produced by Septoria osmorhizae Pk. on Osmorhiza longistylis (Torr.) D. C. The leaf of *Osmorhiza longistylis* is very thin. The upper

epidermal cells are large and oval to elongate in shape, while those of the lower epidermis are uniformly narrow and much elongated. There is no well-defined palisade parenchyma. The cells of the spongy parenchyma vary from oval to elongate in shape and are arranged parallel to the epidermis.

The lesions on the leaf are small and ovate to angular in outline. The central holonecrotic portion is grayish-white in color while the plesionecrotic zone surrounding it is somewhat darker.

The intercellular hyphae are abundant in the holonecrotic area. The cell contents in this portion of the leaf have entirely disappeared and the cell walls have collapsed, resulting in a tangled mass of cell walls and hyphae. Where there are no large veins the hyphae can be traced for some distance into the plesionecrotic zone and the tips of the advancing hyphae are not more than two or three cells removed from normal tissue. The cells of this region are in various stages of disorganization, depending on the distance they are removed from the holonecrotic area. In no case was there any evidence of cicatrice formation.

Lesions produced by Cercospora caulophylli Pk. on Caulophyllum thalictroides (L.) Michx. The leaf of this suscept is very thin. The upper epidermal cells vary greatly in size and shape, some being very long with the outer and inner walls almost parallel, while others are oval to almost globose. The outer walls are slightly thickened. Except that the cells are smaller and somewhat thinner-walled, the lower epidermis is similar in its structure. The palisade parenchyma consists of a single layer of short, rather broad cells, more or less rectangular in outline. The entire contents of these cells stain deeply with haemotoxylin. The spongy parenchyma cells are globose to elongate in shape and are loosely arranged in such a manner as to enclose large air spaces.

The lesions in these leaves vary in size and are somewhat angular and irregular in outline. The holonecrotic area is dark brown in color, although in many of the larger lesions this may be a lighter brown in the center. The margin is clear-cut and is surrounded by a yellowish-green plesionecrotic zone of varying width. In some cases the lesions may coalesce to form large, irregular-shaped areas.

A study of a cross-section through one of these lesions shows that in the holonecrotic area the epidermal cells and the cells of the spongy parenchyma have completely collapsed while in the cells of the palisade parenchyma this collapsed condition is only partial. In the plesionecrotic zone the cell contents are more or less disorganized but the walls have not collapsed. In this region the intercellular hyphae can be traced to within two or three cells of normal tissue. Apparently there is no histologic reaction which would in any way hinder the advance of the pathogene.

Lesions produced by Cercospora symplocarpi Pk. on Symlocarpus foetidus (L.) Nutt. The upper epidermal cells of *Symplocarpus foetidus* are large and for the most part much longer than broad, with the outer walls fairly straight and the inner ones curving strongly downwards. Both the outer and radial walls are slightly thickened. The lower epidermis is similar to the upper except that the walls are scarcely thickened. The palisade layer is open in structure and consists of a single layer of very short, broad, rectangular cells. Just beneath the palisade layer and making up a part of the spongy parenchyma are two or three rows of cells varying in size and shape but placed for the most part with their long axes parallel to the epidermis. The remainder of the spongy parenchyma is made up of chains of thin-walled cells, irregular in shape and size, and arranged in such a manner as to enclose large air spaces. These cells contain relatively few chloroplasts.

The lesions on the leaf are large, oval in shape, and irregular in outline save where they are bounded by veins. The holonecrotic center is brown in color, shading into the dark brown or black plesionecrotic border. This border is often somewhat raised and is sharply outlined.

A study of a cross-section through one of these lesions (Fig. 6) shows a general collapse of the cells in the holonecrotic area. The plesionecrotic zone is very narrow and consists at most of three or four cells. The nuclei and chloroplasts have largely disappeared from these cells but apparently there has been no shrinking of the walls. In some cases the upper epidermal cells for some distance beyond the necrotic area have collapsed but the cells beneath are apparently normal. No trace of the hyphae was found beyond the holonecrotic region.

In the case of artificial wounding, the cells a short distance back from the wound have entirely collapsed. At the inner edge of this area there is a slight enlargement of the cells of the palisade parenchyma and of those cells lying just beneath it. The walls of these enlarged cells have become suberized, as have also the walls of the epidermal cells bordering upon this area.

Lesions produced by Cercospora menispermi Ell. et Hollw. on Menispermum canadense L. The leaf of *Menispermum canadense* has a heavy upper epidermis composed of large cells with much thickened outer walls. The lower epidermis is also made up of large cells but the outer walls are relatively thin. The palisade parenchyma is made up of a single layer of closely packed, elongated cells and a second layer which is more loosely arranged. The spongy parenchyma is very open with large irregular air spaces.

The brownish lesions on the leaf are somewhat irregular in shape and

size and are largely holonecrotic in character. The narrow plesionecrotic margin is purplish-black in color and well-defined.

In the holonecrotic area the leaf is somewhat thinner than normal. This is due to the collapse of the epidermal cells and those of the spongy parenchyma. The cells of the palisade parenchyma are much reduced in length and are either empty or partially filled with a brownish substance. The intercellular hyhpae are abundant in this region but apparently do not extend into the plesionecrotic zone. There is no change in the character of the cells at the edge of the lesion indicating the formation of a cicatrice.

Lesions produced by Cercospora circumcissa Sacc. on Prunus serotina Ehrh. The upper epidermis of *Prunus serotina* is somewhat thicker than is the lower but otherwise they are very similar. Both the outer and inner walls of these cells are considerably thickened. The palisade parenchyma

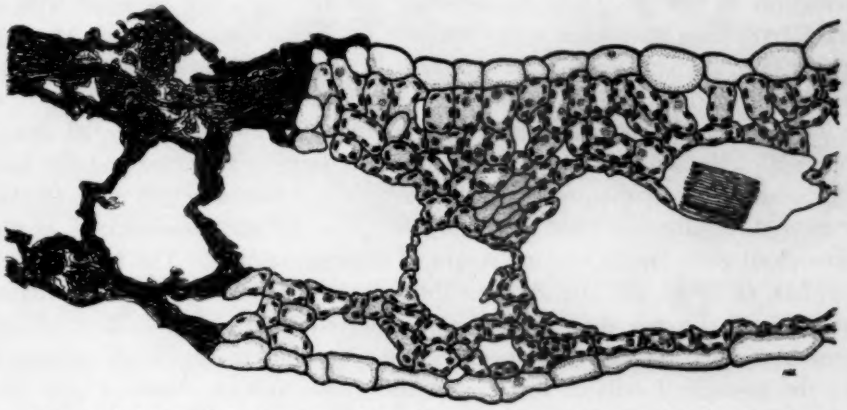

FIG. 6. Cross section through a diseased leaf of *Symplocarpus foetidus* showing the histologic changes induced by *Cercospora symplocarpi*. ×197.

consists of a single layer of cells which are somewhat elongated and taper slightly toward the lower end. These cells are rather compactly arranged, although there are evident intercellular spaces between many of them. The cells of the spongy parenchyma are irregular in size and shape and are so arranged that they enclose large air spaces.

Lesions produced on this suscept are angular in outline and quite variable in size. The holonecrotic portion varies in color from a light brown to a gray and is bordered by a reddish-brown plesionecrotic zone.

In the holonecrotic area the leaf is somewhat thinner than normal. The contents of the cells of the spongy parenchyma have disappeared and the walls have collapsed. This is also the case in many of the palisade cells, but some are partially filled with a dense granular substance. The hyphae are not at all plentiful in any part of the lesion, but occasional threads are

visible between the cells in both the holonecrotic and the plesionecrotic areas. The contents of the cells of the latter region are more or less disorganized. There was no evidence of cicatrice formation at the edge of the lesion.

Lesions produced by Ramularia primulae Thüm. on Primula polyantha Mill. The upper epidermis of *Primula polyantha* is much thicker than is the lower. It is made up of much larger cells, the outer walls of which are markedly thickened. Occasional cells in both of these layers are filled with a dense, tannin-like substance. The palisade parenchyma is made up of a single layer of short thick cells, loosely arranged. The spongy parenchyma is composed of cells of various sizes and shapes and is moderately open in arrangement.

The lesions on the leaf of this suscept are variable in size and somewhat irregular in outline. The holonecrotic center is grayish in color with a sharply defined somewhat darker margin. Surrounding this area there is a rather broad yellowish-green plesionecrotic zone. The lesions are numerous upon the leaf and often unite to form large necrotic patches.

In the holonecrotic area the cell contents have broken down and disappeared. The cell walls have collapsed to a large extent, leaving the leaf somewhat thinner than normal at this point. In the outer edge of this region the epidermal cells are collapsed (Fig. 7) and the contents of the mesophyll cells are in various stages of disorganization. The intercellular hyphae, although not abundant in the holonecrotic area, are plainly visible and extend through the plesionecrotic zone to a point within a few cells of normal tissue. In the plesionecrotic zone none of the cells are collapsed. In the mesophyll cells of this region the nuclei and chloroplasts have disappeared and the cytoplasm is partially broken down. There is no evidence of any healing tissue at or near the edge of the lesion which would tend to prevent the advance of the pathogene.

Lesions produced by Ramularia ranunculi Pk. on Ranunculus recurvatus Poir. The leaf of *Ranunculus recurvatus* is extremely open in structure. The upper and lower epidermis are very similar in structure and are composed of rather irregularly shaped cells whose outer walls are slightly thickened. There is no well-defined palisade parenchyma, but the irregularly shaped cells which compose the mesophyll are more closely arranged in that region.

The lesions on the leaves of this suscept are large, circular to oval in outline except where they are bounded by large veins. They are entirely holonecrotic in character and brownish in color with somewhat darker centers.

In the center of the diseased area the contents of all of the cells have disappeared and the walls have collapsed, thus causing the leaf to be much

thinner than normal at this point. Farther out toward the edge of the lesion the epidermal cells may or may not be collapsed and the leaf gradually increases to normal thickness although the contents of the mesophyll cells may be entirely disorganized and the walls partially shrunken. The threads of hyphae can be seen in the intercellular spaces of the mesophyll and extend to the very edge of normal tissue. There is no evidence of any suscept reaction of any kind which would tend to prevent the advance of the pathogene.

Lesions produced by Ramularia decipiens Ell. et Ev. on Rumex crispus L. The leaf of *Rumex crispus* is very open in structure. The cells of the upper epidermis contain a dense granular substance in the protoplasmic layer, and in some cases the cells are completely filled with this material. The palisade parenchyma is composed of a single layer of rather short, broad

FIG. 7. Cross section through a diseased leaf of *Primula polyantha* showing the histologic changes induced by *Ramularia primulae*. ×187.

cells, loosely arranged. The cells of the spongy parenchyma vary widely in size and shape.

The lesions vary greatly in size and are more or less irregular in shape. The central part of the holonecrotic area is light brown in color with a wide, somewhat darker-colored margin. This is usually surrounded by a purplish plesionecrotic zone.

The holonecrotic area is very little thinner than normal. The cell contents in this part of the leaf have entirely disappeared. The epidermal cells, and to a certain extent the cells of the spongy parenchyma, have collapsed. The hyphae are intercellular and can be traced throughout the plesionecrotic zone to the very edge of normal tissue. In this zone the cells of the epidermis and the mesophyll are in various stages of disorganization and collapse. There is no evidence of histologic changes which would tend to prevent the advance of the pathogene.

Lesions produced by Alternaria solani (E. et M.) Jones et Grout on Solanum tuberosum L. The structure of the healthy leaf of *Solanum tuberosum* is rather open. The upper epidermis is much thicker than the

lower and the cells are more or less filled with some granular substance. The outer walls of the cells of both the upper and lower epidermis are slightly thickened. The cells of the palisade parenchyma are narrow, much elongated, and irregular in outline with large air spaces between the individual cells. The cells of the spongy parenchyma are irregular in shape, and the air spaces in this tissue are also large.

The lesions on this suscept are entirely holonecrotic in character, irregular in shape and size, somewhat angular in outline and marked with concentric rings. The color is dark brown and the margin is well defined.

In the diseased area the upper epidermal and palisade cells are filled with a dense granular substance (Fig. 8). The cells of the spongy paren-

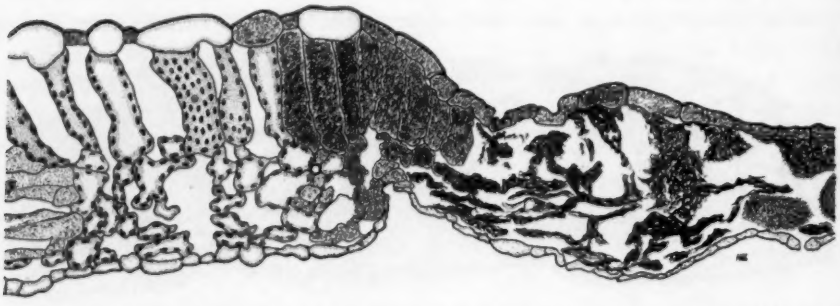

FIG. 8. Cross section through a diseased leaf of *Solanum tuberosum* showing the histologic changes induced by *Alternaria solani*. ×145.

chyma are collapsed, as are also the cells of the lower epidermis. The palisade cells are much reduced in length and partially collapsed except in the region of the concentric rings where they stand out more prominently. The necrotic area ends almost abruptly in normal tissue, although the cells on the outer edge have not yet collapsed. The hyphae are abundant between the shrunken mesophyll cells, particularly in the spongy parenchyma, and can be traced almost to the edge of the normal tissue. There is no evidence of cicatrice formation.

Lesions produced by Alternaria solani (E. et M.) Jones et Grout on Lycopersicum esculentum Mill. The general structure of the leaf of *Lycopersicum esculentum*, the appearance of the lesions, and the reaction of the suscept to the invasion of the pathogene are almost identical with those just described for *Solanum tuberosum*.

Lesions produced by Macrosporium cucumerinum Ell. et Ev. on Cucumis melo L. The leaf of this suscept is quite thick. Both the lower and upper epidermal cells are large and their outer walls are slightly thickened. The palisade parenchyma is made up of a single layer of narrow, much

elongated cells, rather closely arranged. The cells of the spongy parenchyma are variable in shape and size and are arranged somewhat loosely.

The lesions caused by this pathogene are variable in size, regular in outline except where they are bounded by veins, and frequently coalesce to form large necrotic areas. The holonecrotic center is light brown in color. A narrow plesionecrotic zone surrounds this area but is more marked on the under surface of the leaf than on the upper.

In the holonecrotic area the leaf is much thinner than normal, the cell contents have disappeared, and the walls have collapsed. This collapsing of the walls is more complete in the cells of the spongy parenchyma. These shrunken cells are interwoven with the intercellular hyphae and they are present in the plesionecrotic zone, extending to a distance of one or two cells from normal tissue. The contents of the cells in this zone are partially broken down but the walls have not collapsed.

Lesions produced by Phyllosticta fraxinii Ell. et Mart. on Fraxinus americana L. In the leaves of *Fraxinus americana* the upper and lower epidermis are quite similar in structure except that in the former the cells are filled with some dense granular substance. The palisade parenchyma is made up of a double layer of narrow, elongated cells which are arranged to form a compact wall. The cells of the spongy parenchyma vary in size and shape and are rather closely arranged, the intercellular spaces being comparatively small.

The lesions on this leaf are large and more or less irregular in outline. The main body of the lesion is holonecrotic in character and light brown in color. It is surrounded by a purplish-black plesionecrotic zone of varying width.

In the holonecrotic area the epidermal cells and the cells of the spongy parenchyma are empty and collapsed. The palisade cells are slightly reduced in length, somewhat shrunken, and partially filled with some dense granular material. The leaf at this point, however, is not much thinner than normal. The contents of the cells in the plesionecrotic zone are somewhat disorganized. The hyphae are visible between the cells in this region and apparently advance to within two or three cells of healthy tissue.

Lesions produced by Pseudopeziza medicaginis (Lib.) Sacc. on Medicago sativa L. Both the upper and lower epidermis of *Medicago sativa* are similar in structure, the cells varying considerably in size and shape, with the outer walls markedly thickened. The palisade cells are arranged in one or two layers, some being rather short and broad while others are much elongated. They are somewhat open in arrangement. The cells of the spongy parenchyma are also loosely arranged and vary greatly both in size and shape.

The lesions are numerous and scattered on the leaves. They appear as small holonecrotic areas which are dark purple in color and surrounded by a yellowish plesionecrotic border which blends into the normal green of the leaf.

In the holonecrotic area the palisade cells are almost entirely replaced by the stroma of the fruiting body while the cells of the spongy parenchyma are either collapsed or filled with the hyphae. In the plesionecrotic zone the epidermal cells may be collapsed. The hyphae can frequently be seen in one or more cells beyond this point and occasional threads of hyphae can be seen in the mesophyll cells of this region and may extend to within two or three cells of normal tissue. The cells in this zone have their contents more or less disorganized and an occasional palisade cell is filled with some dense brownish substance, but there is little if any collapsing of the cell walls. There is no evidence of cicatrice formation.

Lesions produced by Pseudopeziza medicaginis (Lib.) Sacc. on Medicago lupulina L. The structure of the leaf of *Medicago lupulina* differs from that of *Medicago sativa* in being very much thinner. This difference in thickness is mainly due to the shorter palisade cells but is also due to some extent to a thinner layer of spongy parenchyma.

The lesions on this suscept are very similar in their general characters to those described on *Medicago sativa.* The suscept reactions and the extent of the mycelial invasion are in all respects similar to those just described for *Medicago sativa.*

Lesions produced by Pseudopeziza trifolii (Bernh.) Fckl. on Trifolium pratense L. The leaf of *Trifolium pratense* resembles that of *Medicago sativa* in its general structure. The palisade cells are not so long, however, and although the leaf blade is approximately the same thickness in the two species, the lack in length of palisade cells is made up by a corresponding increase in thickness of spongy parenchyma in *Trifolium pratense.*

In general the character of the lesions on the leaves is similar to that given for the lesions on leaves of *Medicago sativa.* The suscept reactions and the extent to which the hyphae have penetrated the leaf tissues are the same as have been described in connection with *Medicago sativa.*

Lesions produced by Pseudopeziza ribis Kleb. on Ribes grossularia L. The leaf of *Ribes grossularia* is rather thick. The upper epidermis differs from the lower in having larger cells whose walls are somewhat thicker. The palisade parenchyma consists of two or even three layers of cells. These cells are quite broad and those of the upper layer are much longer than those of the lower. They are closely packed together. The cells of the spongy parenchyma are variable in size and shape. They are arranged in chains or groups and, while only moderately open in arrangement, the intercellular spaces are at times quite large.

The lesions on this suscept are small, more or less round in outline with an indefinite margin. The color is a dark purple with a grayish center in the older lesions.

The condition existing here as a result of the invasion of the pathogene is so similar to that already described for *Pseudopeziza medicaginis* that no further description is necessary.

Lesions produced by Rhytisma ilices-canadensis Schw. on Ilex verticillata (L.) Gray. A study of the leaf of *Ilex verticillata* shows the upper epidermal cells to be very regular in shape and size, almost rectangular in outline, and with a heavy outer wall. The lower epidermal cells are not so regular either in shape or size as those of the upper epidermis, nor are the outer walls quite so thick. The palisade parenchyma consists of a single layer of cells. These are narrow, much elongated, very regular in shape, and closely arranged. The cells of the spongy parenchyma are irregular in size and shape and so arranged as to leave rather large air spaces between the cells or groups of cells.

The lesions on the leaves are irregular in size and shape. The holonecrotic portion is black in color and is surrounded by a narrow, light green plesionecrotic border which blends into the normal green of the leaf.

When viewed in cross-section the black portion of the lesion is seen to consist of a stroma with a very dense black outer covering. On the upper surface of the leaf this stroma is only slightly elevated and beneath it the palisade cells are still in evidence, although they have shrunken and the contents have become disorganized. The hyphae are apparently intercellular. On the lower surface of the leaf the stroma is very much thicker and in some cases may cover a larger area than does the upper stroma. The contents of the spongy parenchyma cells which lie between the two stromata are usually wholly disorganized. Where the lower stroma covers a larger area than the upper, those parenchyma cells lying next to the stroma are disorganized while the remaining mesophyll cells may be perfectly normal. The hyphae do not appear to extend into the plesionecrotic zone and the contents of the cells in this region are only partially disorganized. There are apparently no histologic changes in the suscept which would prevent the further advance of the pathogene.

Lesions produced by Rhytisma ilices-canadensis Schw. on Nemopanthus mucronata (L.) Trel. Although similar in its general structure, the leaf of *Nemopanthus mucronata* is much thinner than that of *Ilex verticillata* and the upper epidermal cells are partially filled with a dense material resembling tannin.

The lesions are very similar in appearance to those just described on *Ilex verticillata* and in general the suscept reaction is the same. In the

sections examined, however, the hyphae extend a short distance into the plesionecrotic zone.

Lesions produced by Rhytisma acerinum (Pers.) Fr. on Acer rubrum L. The general structure of the leaf of *Acer rubrum* is the same as that of *Nemopanthus mucronata*. The appearance of the lesions and the type of suscept reaction are very similar to those described for *Ilex verticillata*.

Lesions produced by Mycosphaerella fragariae (Tul.) Lind. on Fragaria virginiana Duch. In the healthy leaf of this suscept the cells of both epidermal layers are large and rather broad. Many of the cells of the upper epidermis are partially filled with a material resembling tannin. The palisade parenchyma is composed of a single layer of compactly arranged cells which are rather short and taper slightly towards the lower end. The cells of the spongy parenchyma are irregular in size and shape and are very loosely arranged.

The lesions on the leaf are somewhat irregular in size and shape. In the older lesions the center of the holonecrotic area is grayish in color with a reddish-purple margin. This is surrounded by a wide plesionecrotic zone which is also reddish-purple in color, blending into the normal green of the leaf.

In the center of the holonecrotic area the cells have collapsed and the leaf is somewhat thinner than normal at this point. In the outer edge of this area the cells are filled with a brownish substance resembling tannin which failed to react to any of the stains used. These cells are not collapsed. In the plesionecrotic zone all of the epidermal cells are filled, or at least partly filled, with some material which stains deeply with haemotoxylin. The contents of the mesophyll cells of this region stain more intensively than do the normal cells. The chloroplasts, although present, appear to be degenerating. The hyphae are present between the cells in the holonecrotic area but no trace of them can be found beyond the zone of mesophyll cells which are filled with the tannin-like substance. In no case is there any evidence of cicatrice formation although occasional cells at the outer edge of the holonecrotic area may be slightly enlarged.

When leaves of *Fragaria virginiana* are wounded a cicatrice is formed about the edge of the wounded portion. Multiplication and enlargement of the mesophyll cells occurs. All of these cells are thick-walled and the walls of the outer row are suberized. There is no evidence of a wound periderm such as occurs in *Prunus sp.*, but all of the cells are filled with the same granular substance found at the edge of the lesions produced by *Mycosphaerella fragariae*.

Lesions produced by Mycosphaerella grossulariae (Fr.) Lind. on Ribes prostratum L'Her. In a healthy leaf of *Ribes prostratum* the upper epi-

dermis is somewhat thicker than the lower. The walls of the upper epidermal cells are considerably thickened, and this thickening is present to a lesser extent in the walls of the lower epidermal cells. The palisade parenchyma is well-defined and is made up of a single layer of elongated cells closely packed together. The spongy parenchyma is composed of cells of various shapes and sizes which are loosely arranged.

The lesions on this suscept are small and rather regular in outline. The holonecrotic center is grayish-white in color and is bordered by a narrow purplish-black plesionecrotic zone.

The holonecrotic portion is very little thinner than is the normal portion of the leaf. In this area many of the cells, both in the palisade and in the spongy parenchyma, have become filled with a dense granular substance resembling tannin. Such cells have only partially collapsed. All other cells in this area are apparently empty. The epidermal cells are collapsed and this condition is quite common even in the plesionecrotic zone. The contents of the mesophyll cells in this zone are becoming disorganized and the cells are filling up with the dense material already referred to. The intercellular hyphae extend for a short distance into the plesionecrotic zone. There is no evidence of the formation of a healing tissue about the edge of the lesion.

Lesions produced by Sphaerella tussilaginis Rehm. on Tussilago farfara L. In the healthy leaf of *Tussilago farfara* the upper epidermis is much thicker than the lower, the cells are larger, and the outer walls are much thicker. While the upper epidermal cells are arranged above the palisade cells in the ordinary way, the lower epidermis is decidedly wavy in its appearance. It appears as a chain of cells attached to the spongy parenchyma at regular intervals, each loop of the chain forming the outer boundary of a large air space. The palisade parenchyma is composed of about four layers of cells, the cells of the upper layer being much shorter and more regular in shape than those of the lower layers. These cells are closely packed, no intercellular spaces being visible in cross section of the leaf. The cells of the spongy parenchyma vary from almost square to very narrow and much elongated and are arranged in chains or groups. To the lower part of these chains, or groups of cells, the lower epidermis is attached.

The lesions on the leaf are circular in outline except when they are bounded by large veins. In the older lesions the holonecrotic center is grayish in color, and this is surrounded by a dark purple plesionecrotic zone of varying width. The edge of the lesion is not well-defined.

In the holonecrotic area the cells are entirely empty and the cell walls have collapsed. The leaf is about one half its normal thickness at this point.

In the plesionecrotic zone the chloroplasts have broken down and disappeared but otherwise there seems to be little disorganization of the cell content. The upper layer of palisade cells and the cells of the upper epidermis contain some substance which stains deeply with haemotoxylin. No trace of the fungus was found outside of the holonecrotic area nor was there any evidence of cicatrice formation.

Lesions produced by Gnomonia leptostyla Fr. on Juglans cinerea L. The leaf of *Juglans cinerea* is very thin. The majority of the cells of the upper and all of those of the lower epidermis are narrow, long, and irregular in outline. In the upper epidermis, however, are found either single cells or groups of cells which are much larger than the others and either globose or oval in shape. The palisade parenchyma consists of a single layer of cells which are rather short and slightly narrowed at the lower end. The spongy parenchyma is made up of two or three layers of loosely arranged cells. The cells of the upper layer are large and resemble those of the palisade layer while the cells of the lower layers are very narrow and elongated.

The lesions on the leaf are somewhat variable in size and frequently unite to form large necrotic areas. The holonecrotic area is brownish-gray in color. The extreme margin of this area is really plesionecrotic in character.

In the holonecrotic area the leaf is very little thinner than normal. The epidermal cells and those of the spongy parenchyma are empty and the walls have collapsed. The palisade cells are partially filled with some dense granular material. These cells have apparently been reduced in length but the walls have not collapsed to the same extent as have the walls of the other mesophyll cells. In the plesionecrotic zone the contents of the cells are only partially disorganized. The hyphae are not abundant in the diseased region, but occasional threads are visible between the cells in the holonecrotic area and these extend to some extent into the plesionecrotic zone. This zone blends gradually into normal tissue.

Lesions produced by Guignardia bidwellii (Ell.) V. et R. on Ampelopsis tricuspidata Planch. In the healthy leaf of *Ampelopsis tricuspidata* the upper epidermis is composed of large cells which are, for the most part, somewhat oval in outline. The outer walls are very thick and the radial and inner walls, while somewhat thinner, are relatively thick as compared with the walls of the mesophyll cells. The cells of the lower epidermis are more irregular in size than those of the upper and the walls are markedly thickened. The palisade parenchyma is well-defined and is made up of two layers of cells. Those of the upper layer are long, narrow, and closely packed, while those of the lower layer are considerably shorter, somewhat

broader and more loosely arranged. The cells of the spongy parenchyma are variable both in size and shape. Some are almost rectangular in outline, others are almost globose, while still others are narrow and much elongated. These cells are rather loosely arranged and the intercellular spaces are fairly large.

The lesions on the leaves are variable in size and are usually irregular in shape. They frequently coalesce to form large necrotic areas and in extreme cases the greater part of the leaf may be affected. The holonecrotic center is brown in color with a sharply defined, lightly raised, purple-black margin which is plesionecrotic in character.

In the holonecrotic area the palisade cells have not collapsed completely but are much reduced in length. Many of these cells are filled with a dense granular material resembling tannin. The cells of the spongy parenchyma are for the most part empty and shrunken. In the plesionecrotic zone the cells have lost their chloroplasts although the cytoplasm is still in evidence (Fig. 9). In this region the cells of the upper layer of palisade parenchyma are slightly longer than normal and the upper epidermal cells are greatly enlarged. In the latter cells the contents have either disappeared or they are densely granular. The remainder of the mesophyll cells and those of the lower epidermis are apparently normal in size although their contents are somewhat broken down. As considerable difficulty was experienced in staining the mycelium, no satisfactory evidence was secured relative to the abundance of the hyphae in the holonecrotic area nor the distance to which it had penetrated the plesionecrotic zone.

Artificial wounding of the leaves of this suscept resulted in the formation of a definite cicatrice (Fig. 10). This cicatrice is of the same general type as that produced on the leaves of *Prunus domestica* when similarly treated.

Discussion

While the leaf tissues of the suscepts included in this group do not respond to the invasion of the pathogene by the formation of a definite cicatrice, nevertheless the cells about the holonecrotic area are far from being normal in character. The changes occurring in these cells are mainly disorganization of the protoplasm and the collapse of the cells. The extent or degree of such changes varies in different plants and seems to be associated with the character of the suscept rather than the pathogene invading the tissues.

In some leaves the cells in the holonecrotic area may be entirely empty and collapsed, a condition found in *Apium graveolens* when attacked by *Septoria petroselini*, and as a result this portion of the leaf may be much

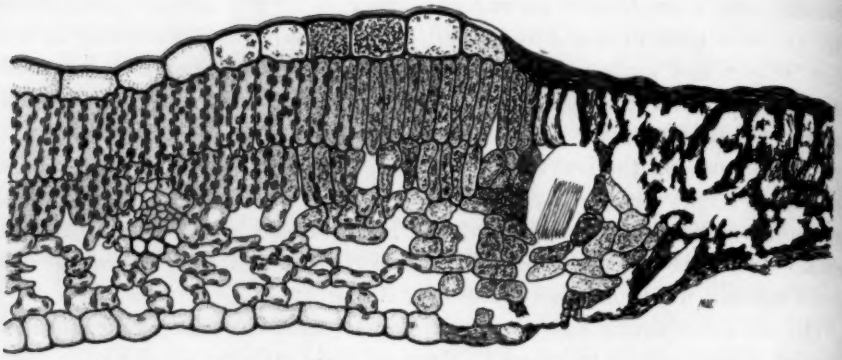

FIG. 9. Cross section through a diseased leaf of *Ampelopsis tricuspidata* showing the histologic changes induced by *Guignardia bidwellii*. ×230.

thinner than normal. On the other hand many of the cells in this area may be partially or wholly filled with a dense granular material as is the case with leaves of *Steironema ciliatum* attacked by *Septoria conspicua*. In such leaves many of the palisade cells and some of the cells of the spongy

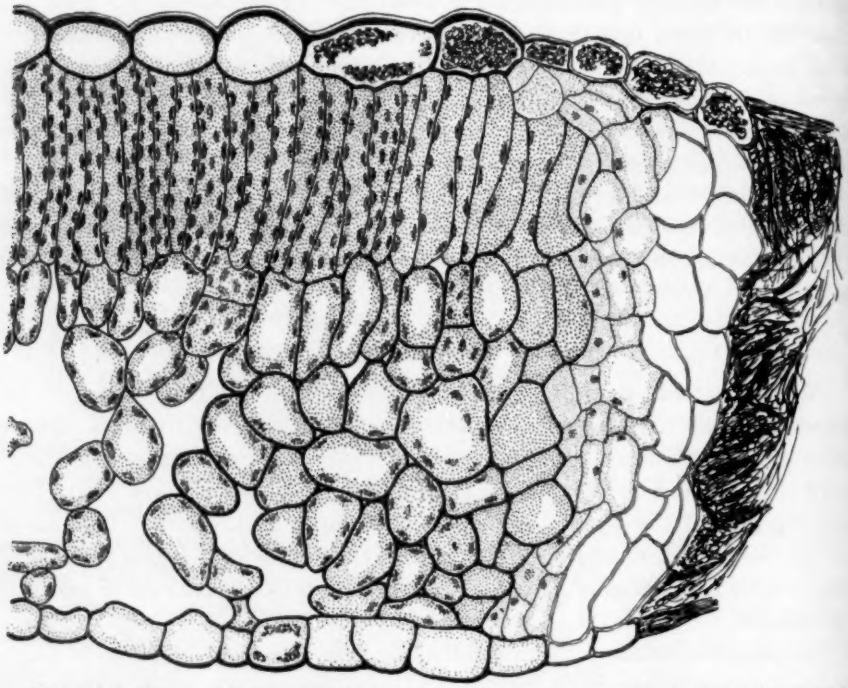

FIG. 10. Cross section through the edge of a wound on the leaf of *Ampelopsis tricuspidata* showing the cicatrice formed under these conditions. ×321.

parenchyma contain this substance and as a result little or no shrinking of the necrotic tissues has occurred.

The extent to which the hyphae of the pathogene penetrate beyond the holonecrotic area differs greatly in the leaves of the plants studied. Not only does this vary with the different plants but it varies in different lesions on the same leaf and is affected in many cases by the presence or absence of veins. The extent of this hyphal invasion is important in its effect upon the cells in the invaded region for such cells invariably show the influence of the pathogene by a disorganization of the protoplasm, followed later by a partial or total collapse of the cells. In leaves of *Apium graveolens* attacked by *Septoria petroselini* the hyphae extend for a long distance beyond the holonecrotic area, as reported by Rogers (16), and the cell contents in this region are more or less disorganized. The same condition is found in leaves of *Primula polyantha* attacked by *Ramularia primulae*, and also in leaves of *Caulophyllum thalictroides* attacked by *Cercospora caulophylli*. On the other hand in leaves of *Symplocarpus foetidus* attacked by *Cercospora symplocarpi* the hyphae extend little if any beyond the holonecrotic area and only a few cells can be classed as plesionecrotic. A similar condition exists in leaves of *Ilex verticillata* attacked by *Rhytisma ilices-canadensis*, in leaves of *Medicago sativa* infected by *Pseudopeziza medicaginis*, and in leaves of *Solanum tuberosum* bearing lesions caused by *Alternaria solani*.

In the case of the last mentioned suscept it was thought that possibly the target-board effect caused by *Alternaria solani* might be due to the formation of a periderm as was shown by Hesler (6) to be the case in *Pyrus malus* attacked by *Sphaeropsis malorum*. Jones (10) and Rands (15) have suggested that this effect is caused by the epidermal cells being thrown into folds due to shrinking caused by the collapse of the mesophyll cells. Whatever may be the effect of this shrinkage of the mesophyll cells the writer's observations show that, for some unknown reason, the palisade cells of the target board lines are only partially collapsed while this collapse is complete in the cells between these lines.

CONCLUSIONS

The results reported in this paper show clearly that whatever may be the factor or factors governing the limitation of the necrosis caused by fungi in the leaf spot diseases of plants, it is by no means due in all cases to the formation of a cicatrice or healing tissue within the suscept, which limits the advance of the pathogene. In some plants the usual reaction of the leaf to pathogene injury appears to be the formation of a cicatrice. In a large majority of cases, however, there is no attempt on the part of the

suscept to lay down such a barrier. Neither does it follow that a plant which forms a cicatrice about mechanical wounds in its leaves will respond in the same way to localized necrosis caused by fungi. On the other hand all of the suscepts studied during these investigations which did produce a cicatrice about a fungous necrosis also formed a cicatrice about mechanical injuries. Whether these plants react in the same way to the attacks of other leaf-spotting organisms remains a problem for future work.

SUMMARY

Leaves of *Prunus domestica, P. avium, P. cerasus,* and *P. virginiana,* when attacked by species of *Coccomyces,* form a definite cicatrice about the edge of the lesion, thus isolating the diseased portion from the healthy. The same reaction occurs when leaves of *Pyrus communis* are attacked by *Mycosphaerella sentina* and when leaves of *Beta vulgaris* are attacked by *Cercospora beticola.* Artificial wounding of leaves of these plants results in the formation of a cicatrice similar to that formed in diseased leaves.

No cicatrice is formed in either diseased or wounded leaves of *Podophyllum peltatum* or *Symplocarpus foetidus.* In wounded leaves of these plants the cells for some distance from the edge of the wound are dead and collapsed while the walls of a few cells adjoining the inner edge of this collapsed area are suberized.

Diseased leaves of *Fragaria virginiana* showed no evidence of cicatrice formation, but wounded leaves of this plant formed a definite cicatrice about the edge of the wound. These cells have thickened walls and are suberized in part while all of the cells are filled with a dense granular material. There is no evidence of a wound periderm such as is formed in wounded leaves of *Prunus domestica.*

Although diseased leaves of *Ampelopsis tricuspidata* do not react by the formation of a cicatrice, yet wounded leaves of the same plant form a well developed wound periderm about the edge of the injury.

Diseased leaves of all other suscepts included in this investigation failed to form a cicatrice separating the lesion from healthy tissues. The type of reaction in these cases consists of a degeneration of the protoplasm, with or without granular deposits, together with more or less collapsing of the cells. No evidence of suberization of the walls of the cells at or near the edge of the lesions was observed. These leaves were not wounded and their reaction to such injuries is unknown.

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LITERATURE CITED

1. BLACKMAN, F. F., and G. L. C. MATTHAEI. On the reaction of leaves to traumatic stimulation. Ann. Bot. **15**: 533–546. 1901.
2. BRETFELD, VON F. Ueber Vernarbung und Blattfall. Jahrb. wiss. Bot. Pringsheim. **12**: 133–160. 1879–81.
3. CHAMBERLAIN, C. J. Methods in plant histology. 314 pp. Univ. Chicago Press. 1915.
4. DURAND, E. J. The differential staining of intercellular mycelium. Phytopath. **1**: 129–130. 1911.
5. FRANK, A. B. Die Krankheiten der Pflanzen. Vol. I. 344 pp. Breslau. 1895.
6. HESLER, L. R. Blackrot, leaf spot, and canker of pomaceous fruits. N. Y. (Cornell) Agr. Exp. Sta. Bul. 379: 53–148. 1916.
7. HIGGINS, B. B. Contribution to the life history and physiology of *Cylindrosporium* on stone fruits. Amer. Jour. Bot. **1**: 145–173. 1914.
8. JACKSON, GEMMA. Crystal violet and erythrosin in plant anatomy. Stain Technology **1**: 33–34. 1926.
9. JAHRMAN, F. Ueber Heilung von Epidermiswunden. Centralbl. Bakt. **37**: 564–595. 1913.
10. JONES, L. R. Various forms of potato blight and their causes; studies upon *Macrosporium solani* E. and M. Vt. Agr. Exp. Sta. Rept. **9**: 72–87. 1895.
11. MASSART, J. La cicatrisation chez les végétaux. Mem. Acad. Belg. **57**: 3–68. 1898.
12. MCWHORTER, F. A. The diseases of *Carica papaya*. Cornell Univ. thesis. 67 pp. 1927.
13. MIEHE, H. Ueber Wanderungen des Pflanzlichen Zellkernes. Flora **88**: 105–142. 1901.
14. PIERCE, N. B. A disease of Almond trees (*Cercospora circumcissa* Sacc.). Jour. Mycol. **7**: 66–77. 1892.
15. RANDS, R. D. Early blight of potato and related plants. Wisc. Agr. Exp. Sta. Res. Bul. 42. 1917.
16. ROGERS, S. S. The late blight of celery. Cal. Agr. Exp. Sta. Bul. 208. 1911.
17. SAMUEL, G. On the shot-hole disease caused by *Clasterosporium carpophilum* and on the shot-hole effect. Ann. Bot. **41**: 375–404. 1927.
18. VAN BUESEKOM, JAN. On the influence of wound stimuli on the formation of adventitious buds in the leaves of *Gnetum gnemon* L. Rec. Trav. Bot. Néerland. **4**: 149–175. 1908.
19. VAUGHAN, R. E. A method for the differential staining of fungus and host cells. Ann. Mo. Bot. Gard. **1**: 241–242. 1914.
20. WOIT, MAGDA. Uber Wundreaction an Blättern und den anatomischen Bau der Blattminen. Mitteil. Deutsch. Dendrol. Ges. **35**: 163–187. 1925.
21. WYLIE, R. B. Concerning the capacity of foliage leaves to withstand wounding. Proc. Iowa Acad. Sci. **28**: 293–304. 1921.
22. ———. Some wound responses of foliage leaves. Proc. Iowa. Acad. Sci. **29**: 238–244. 1922.
23. ———. Leaf structure and wound response. Science **65**: 47–50. 1927.
24. WYNEKEN, K. Zur Kenntniss der Wundheiling an Blättern. 63 pp. Inaugural-Dissertation, Göttingen, 1908.

THREE HELMINTHOSPORIUM DISEASES OF SUGAR CANE

JAMES A. FARIS

In 1892 van Breda de Haan (1), a Dutch investigator working in Java, described a leaf disease of sugar cane, which, in its mature stage, causes a red to red-brown spot surrounded by a yellowish zone, the center of the spot drying up as the infection advances. Owing to the elongated elliptical shape of this spot, it was named "eye spot" by Krüger (1).

This disease is further described as elongating length-wise of the leaf in rather wide, yellowish bands from the typical spots, the spots and bands thereupon suggesting a peacock-feather in form. In the end, all the attacked leaf parts dry up and die, but it is still possible to find the original form of the spot again by holding the leaf toward the light, as the tail-like spot will appear lighter than the other parts.

Details are given as to the pathological anatomy of the diseased leaves. It is also pointed out that cane varieties differ greatly in their susceptibility to the disease, the Bali variety being so susceptible that large portions of the leaves are attacked at an early stage and the plants develop very poorly. On other varieties in which disease is limited to a few broad bands, the disease in most cases is not serious.

The influence of weather conditions is stated to be very great, the worst attacks coming at the end of the rainy season, and, under conditions ideal for the attacking fungus, the growth of the plants is slowed up to a considerable degree.

The cause of the disease was shown by infection experiments to be a fungus, which was hyaline outside the leaf tissue but developed a brown tint inside the leaf. The fungus was observed to produce dark brown conidiophores through the stomata; very seldom were they seen breaking through the epidermis to reach the surface. These dark brown conidia bearers were poly-celled and when fully grown measured 120–160 μ. From these, conidia are produced with 6–9 cells measuring 60–80 μ in length by 9–12 μ in breadth. Mature spores are described as having a lengthened oval form and a smoke-brown color. No illustrations are given of this fungus, but the organism was thought by van Breda de Haan to be a new species in the genus *Cercospora* and he named it *Cercospora sacchari.*

In 1894 Lucassen and Went (20), other investigators in Java, published a colored plate of the eye spot disease of sugar cane leaves, which they describe briefly, citing the article by van Breda de Haan for further

reference, and giving the fungus *Cercospora sacchari* van Breda de Haan as the causal organism. Their colored plate is very typical of the eye spot of sugar cane as I have observed it in Porto Rico, Santo Domingo, and Cuba; and specimens of eye spot in Cuba were identified by Hawaiian workers as identical with that of Hawaii. From this it seems clear that the disease described by van Breda de Haan is identical with the eye spot disease of cane which we are studying.

The colored plate of Lucassen and Went was again published in the well-known textbooks by Wakker and Went (23) in 1898 and by Krüger (15) in 1899. Both of these books contain drawings of spores of the eye spot fungus which show it to be a *Helminthosporium* rather than a species of *Cercospora*.

Butler and Hafiz (4) in 1913 reported upon some new sugar cane diseases in India and described a Helminthosporiose of sugar cane leaves at Pusa. Infected leaves are described as first showing "small red spots, which enlarge rapidly, chiefly in a longitudinal direction, and, especially toward the tip of the leaf, may run together to form long streaks. The center of the spot soon changes to a dirty straw color, around which the margin remains red for a time and then becomes dark brown." Reference is then made to a colored plate showing two elongated spots with very irregular borders, with no yellowish halo but with a very narrow, line-like, red border separating a broad, straw-colored center from the green tissues of the leaf. If this spot is typical of the disease these authors had under observation, it could not be mistaken for the eye spot figures by Lucassen and Went (20) and Krüger (15) in Java, by Bruner (2) in Cuba, Cobb (5) and Lee (16) in Hawaii, Cook (8) in Porto Rico, or for the eye spot disease we have observed on sugar cane in Porto Rico and Santo Domingo, and which is being studied at present in Cuba. Butler and Hafiz (4) state also that the spots of their Helminthosporiose occur equally on the thin part of the leaf and on the midrib, but in this the disease differs from eye spot, which seldom occurs on the midrib except in heavy infection on very young plants. Lee and Martin (18) state, "the midrib is rarely attacked (by the eye-spot fungus), if at all, in the case of No. 109." Butler and Hafiz (4) then described the fungus as having sporophores 100–190 μ in length by 5.5–7.5 μ in diameter bearing spores 3–10 septate and 35–60 μ in length by 8.5–12 μ in diameter, and named it *Helminthosporium sacchari*. The following statement is then added: "A fungus which, from published descriptions, bears a considerable resemblance to the above, is known in Java and Hawaii under the name of *Cercospora sacchari* Br. de Haan. It was first described by Breda de Haan in Java, as the cause of a leaf disease to which the name

'eyespot' was given. . . . From figures published in Wakker and Went's well-known textbook of sugar cane diseases, it appears probable that this fungus is a *Helminthosporium* and not a *Cercospora*. A comparison of the two fungi has not been possible and could alone settle the question of their identity."

In 1917, Johnson and Stevenson (13) made *Helminthosporium sacchari* Butler a synonym of *Cercospora sacchari* van Breda de Haan. The fungus was then described as having conidiophores 120–160 μ long bearing spores 32–90 μ × 9–14 μ. No mention was made of a comparative study of the fungi, such as Butler and Hafiz (4) believed "could alone settle the question of their identity." In a later publication these authors, in collaboration with Ashby, Bancroft, and Nowell (14) do not seem to have been certain of the identity of *H. sacchari* Butler and *Cercospora sacchari* Br. de Haan, as they add, after quoting Butler's description of the former, "Probably identical with *Cercospora sacchari*, Br. de Haan, which from illustrations does not appear to be a *Cercospora* but an *Helminthosporium*."

The organism causing eye spot in Porto Rico, Santo Domingo, and Cuba agrees fairly well as to conidiophore measurements and spore measurements with those given for *Cerospora sacchari* Br. de Haan.

Likewise measurements by other authors who have given figures for the eye spot organism seem to agree fairly closely with those given by Breda de Haan, as may be seen from table 1.

TABLE 1.—*Spore measurements of Helminthosporium on sugar cane*

Author	Length	Average	Width	Average	Gen. average of spore measurements
Eye Spot Helminthosporium					
Breda de Haan, Java (1)	60–80 μ	70 μ	9–12 μ	10.5 μ	70 × 10.5 μ
Johnston & Stevenson, Porto Rico (13)	32–90 μ	61 μ	9–14 μ	11.5 μ	61 × 11.5 μ
Cook, Porto Rico (6)	45–110 μ	77.5 μ	12 μ	12 μ	77.5 × 12 μ
do (8)	22–92 μ	58.7 μ	6.6–15 μ	11.1 μ	58.7 × 11.1 μ
Drechsler (9)	32–103 μ	71 μ	9–17 μ	14 μ	71 × 14 μ
Faris, Cuba	29–84 μ	68.9 μ	9–21 μ	12.7 μ	69 × 12.7 μ
Helminthosporium sacchari Butl. (3, 4)	35–60 μ	47.5 μ	9.4–12 μ	10.7 μ	47.5 × 10.7 μ

According to these measurements all authors agree that the spores of the eye spot organism are, on the average, much longer than those given for *Helminthosporium sacchari* by Butler and Hafiz (4) and Butler (3). In fact, five of the six measurements given show the average length of the

spores of the *Helminthosporium* causing eye spot to be greater than the maximum length of the spores of *Helminthosporium sacchari* Butler.

In order to make a comparison of the Helminthosporiose of sugar cane in India with the eye spot in Cuba, a fragment of the type material of the former was solicited from Dr. E. J. Butler. None was available, but Dr. Butler referred us to the Hawaiian Sugar Planters' Experiment Station where authentic material from India could be secured. Through the courtesy of the officials of that station, a specimen was obtained of Helminthosporiose on *Saccharum officinarum* from Pusa, India, collected in December, 1924, by Azambullah Kahn. The material was preserved in 4 per cent formalin solution; hence no cultural experiments could be made.

The spots on the material received are of the type described and figured by Butler and Hafiz (4), and resemble, in the early stages, the well-known ring spot more than they do the eye spot. They differ from the ring spot, however, in having a wider ring of red tissue, which Butler and Hafiz (4), and Butler (3) describe as later becoming dark brown. Since the material received is preserved in formalin, too much dependence should not be placed on the color values. The center of the spots are grey and are covered with conidiophores and conidia of *Helminthosporium*. Spores from these spots varied from 9.40 μ to 15.00 μ in width (weighted average 12.54 μ) and in length from 18.80 μ to 71.44 μ (weighted average 52.60 μ) with an average of 5.5 septa.

Taking into consideration the material in hand, the description of the Helminthosporiose of sugar cane by Butler and Hafiz (4), and the characters given by Butler for *Helminthosporium sacchari,* I am brought to the conclusion that the Helminthosporiose and eye spot are two distinct diseases and are caused by different species of *Helminthosporium*.

This conclusion is based upon the following observations: a) the disease pictured with a colored plate by Butler and Hafiz (4) and by Butler (3), a specimen of which I have in hand in no way resembles eye spot in appearance; b) the organism causing eye spot has spores on the average 20 μ longer than those of *H. sacchari* Butler.

Therefore it is a fundamental error to attribute eye spot to *Helminthosporium sacchari* Butler, which causes an entirely different disease and has different mycological characters. As pointed out by Butler and Hafiz (4) and Butler (3), the organism causing eye spot is, without doubt, a *Helminthosporium*. From the present studies it is clear that it is not *Helminthosporium sacchari* Butler.

As the situation now stands the well-known and destructive eye spot disease is due to a species of *Helminthosporium* having fuliginous thin-walled conidia 3–10 septate, measuring 32–110 μ in length (average 69 μ)

by 6.6–20.8 μ in width (average 12.7 μ), which are not identical with those of *H. sacchari* Butler. Nor are they identical with conidia of *H. stenospilum* Dreschler (9), which causes the brown stripe recently described by the writer (10) as a new Helminthosporium disease of sugar cane.

The organism causing eye spot of sugar cane is provisionally named *Helminthosporium ocellum* n. sp.

Helminthosporium ocellum n. sp.

Occurring on the leaves of *Saccharum officinarum,* on many varieties of which it causes spots of a Bordeaux red (Ridg.) with the color of the center very much diluted at the edges, usually surrounded in the younger stages by a marked halo. Conidiophores of a smoky yellow-brown color, 3–8 septate (the septa generally occurring at intervals of 15–45 μ); 3.5–5 μ in width by 145–380 μ in length; points of attachment of successive spores marked by moderately pronounced geniculations.

Conidia subhyaline, light smoky yellow-brown; mature spores typically slightly curved, with the bulge in the middle third of the spore; maximum diameter, usually near the middle, 9–21 μ (weighted average 12.7 μ); length 29–94 μ (weighted average 69 μ); typically 3–10 septate (average 6.7).

Germination is typically by two germ-tubes, one from each end of the conidium.

Habitat—On *Saccharum officinarum,* collected by the author in Cuba, Porto Rico, Santo Domingo. This fungus, isolated from Florida material, was received in pure culture from Dr. Chas. Drechsler, Nov., 1927, and has been collected in Florida by B. A. Bourne (1927). It has also been reported from Hawaii and Java by various authors.

This species of *Helminthosporium* differs from *H. sacchari* Butl. in having longer, lighter-colored conidiophores and conidia, and from *H. stenospilum* Drechsler in having shorter conidia of a much lighter color (Fig. 5). The leaf spots produced by the three species also have very distinctive characters, as may be seen from the description and illustrations.

The Helminthosporium diseases of sugar cane present some important problems for investigation both in regard to their economic importance and from the standpoint of the fungi causing them. A Helminthosporium spot, which has previously been considered an immature stage of the well-known eye spot, has proved to be a distinct disease and was briefly described (10) as brown stripe of sugar cane. Table 2 gives the outstanding characteristics of the three diseases.

The discovery of this error in considering the Helminthosporiose, brown stripe, and eye spot diseases as a single malady raises the interesting ques-

TABLE 2.—*Diagnostic characters of eye spot, helminthosporiose, and brown stripe diseases of sugar cane*

Character of spotting	Eye spot	Helminthosporiose	Brown stripe
	Bordeaux red color, usually with a marked halo in the young stages. In very susceptible varieties elongating into long tail-like streaks from a single spot to the tips of the leaves. The centers of old spots darken to a dark purplish Bordeaux red and the edges to a burnt sienna.	Red border with straw colored center extending into long streaks by the coalescing of various spots. The margin of the spot is red and changes to a dark brown. Very little, if any, halo outside the red border.	Vandyke brown color with a scant yellowish green halo. In very susceptible varieties the spots elongate by increase of the spot itself rather than as tail-like elongations. The color darkens in older spots, the centers of which have a grayish cast.
Conidiophores	Profusely produced on older leaves throughout diseased area, 145–380 μ long, prostrate, simulating a surface mycelium over the peacock-feather-shaped spots. Marked bulbous base, smoky yellow-brown color.	Profusely produced in the grayish center of the spots, 100–190 μ in length, short, stout, erect, rather rigid. Color dark greenish-brown, paler above.	Sparsely produced over the brown area of the stripes. Color dark-olivaceous. Length medium, 120–260 μ, erect, rather rigid, slightly bulbous at base.
Spores	Light smoky yellow-brown, 12.7 μ × 69 μ, typically slightly curved.	Olive-green to brown, 8.5 – 12 μ × 35 – 60 μ, typically cylindrical or long elliptical in shape.	Dark olivaceous color, 17 μ × 84 μ, typically uniform.

tion as to the correctness of reports upon the world distribution of these various cane diseases. See Plate XV.

EYE SPOT IN CUBA

Herbarium material at the Estación de Santiago de las Vegas shows that eye spot was collected on sugar cane in that vicinity April 5, 1915, by J. R. Johnston; February 15, 1918, by S. C. Bruner; and June 12, 1920, by S. C. Bruner.

Bruner (2) has also reported this disease, which he states is of a linear form in the cane in Cuba, measuring from 5 to 12 by 1 to 2 mm.

Both the eye spot and brown stripe diseases are present at Santiago de las Vegas and, since the brown stripe was considered an immature stage of the eye spot until my investigations were reported upon (10) in March, 1927, it is quite likely that this reference to the linear form of the spot refers to brown stripe rather than eye spot.

Owing to the great resistance of Cristalina cane to infection by the eye spot fungus, this disease has been of minor importance in Cuba. However, the introduction from other countries of new varieties which are susceptible to the disease has brought it to the attention of planters. The disease also has been reintroduced with large importations of cane, and we now find it widely distributed over the island. It is present and widely distributed in Oriente Province, present in at least one place in Camagüey and Matanzas Provinces, and in several places in Havana Province. It is present in at least one locality in Santa Clara Province and probably occurs in Pinar del Rio also.

Varietal Susceptibility to Eye Spot

Lee, Martin, Stender, and Barnum in various papers (16–19 and 21) report H 109 cane to be very susceptible to eye spot in Hawaii. Cook (6) in a recent paper reports upon the susceptibility of a number of canes to this disease in experimental studies and field tests, and his results in Porto Rico agree very closely with mine secured during the outbreak of this disease in Cuba in the winters of 1926–1927 and 1927–1928.

The varieties attacked most vigorously were the following Fajardo Central seedlings: F. C. 136, F. C. 214, F. C. 306, and D 109. The last two varieties were being extended to field scale plantings in Oriente Province, Cuba, but, owing to their great susceptibility to this disease, they are not now being extended as field canes. Table 3 compiles results of natural infections in varietal plots in eight different localities. In no case was the widely grown Cristalina cane suffering losses from this disease, although a severe epidemic had developed in recently imported varieties growing in adjacent fields. In all cases the outbreak of the disease was very severe, and it is believed the grouping is a true representation of the reaction which may be expected from the varieties listed under Cuban cultural conditions.

A more extensive list of varieties would be of interest, but these data include all those planted on the eight plantations visited.

Environmental Factors Influencing Infection

The characteristic spots of this disease can be found in affected areas throughout the year but the disease develops very slowly during the periods of hot and dry weather. The cool rainy weather during the winter months usually brings on a severe epidemic in susceptible varieties. During the drier winter months the epidemic gathers force in pockets and valleys where the dew and fog keep the leaves damp until late in the morning. The disease develops vigorously on rapidly growing cane, differing somewhat in this respect from brown stripe which develops better upon cane of slow

TABLE 3.—*Relative susceptibility of cane varieties to eye spot disease in Cuba*

GROUP I.—Varieties which are very susceptible, that is, in which the spots develop very quickly into reddish streaks to the tip or edge of the leaf. The growth is checked, the leaves quickly dry, and sometimes the disease extends throughout the entire top of the plant, resulting in a top rot.

D 109	F C 214
F C 137	F C 306

GROUP II.—Varieties in which spots develop into reddish streaks but which are not so intense in color as in Group I.

Guanica seedling	D 216
H 109	POJ 36
D 433	P R 417
	POJ 100

GROUP III.—Varieties in which spots are produced abundantly but in which, although conditions were the same as for Groups I and II, streaking was absent or very rare.

B 3412	P R 440
B 6308	P R 492
C 35	Sealey seedling

GROUP IV.—Varieties in which mostly spots are produced but not so plentifully as in Group III.

C 553	F. C. 299
General Betancourt	P R 328
Yellow Caledonia	B 3405
Caña Blanca	P R 724
D 1135	P R 725

GROUP V.—Varieties in which spotting is rare, even when growing near heavily infected varieties.

B H 10 (12)	POJ 2714	C 219
B 4596	POJ 2725	P R 700
B 6032	P R 358	P R 16/13
EK 28	S C 12 (4)	P R 707
P R 209	Cristalina	
	Tjepering	

GROUP VI.—Varieties which are resistant and in which very little, if any, spotting was observed. When spots appeared they were small flecks.

D 117	POJ 2727	POJ 36
Java 105	Uba	Kavangire
Java Unknown	Co 281	Cuba 64/21
Badila	D 116	B 1809
POJ 234		B 1753

growth at any time of the year, the outbreaks being much more severe during the dry period of July and August.

Control Measures

It is probably unwise to plant any of the varieties of Group I in Cuba, as a combination of environmental conditions may occur which would cause very serious loss. If canes of Group II are planted, there may be considerable loss in the more humid regions because of the eye spot disease. The fungus is so widely distributed that the susceptibility of any new varieties must be determined before their planting on a field scale can be safely undertaken.

BROWN STRIPE OF SUGAR CANE

During the fall of 1924, a brown linear striping was observed throughout Cuba upon Cristalina cane. From these stripes it was seldom possible to secure sporulating material from the field before the leaves had dried. From spots on dried leaves a *Helminthosporium* could easily be secured. Small blocks of the brown striped areas were taken from green leaves, surface sterilized, and then plated in cornmeal agar. In a very large percentage of cases pure cultures of a *Helminthosporium* were secured, the spores of which were considerably larger than those described by Butler for *H. sacchari.* However, since it was generally stated that the *Helminthosporium* on sugar cane was *H. sacchari* and since the leaf disease under observation resembled to some extent immature stages of the eye spot disease, no serious attention was given to it until late in the season of 1925 and during 1926. During the dry seasons of these two years the outbreak on Cristalina cane, the chief commercial variety of Cuba, was so severe that in some sections of the island both the young and old leaves were thoroughly covered with the characteristic elongated narrow spots or stripes. The variety B H 10 (12) was also badly attacked in the fields of the Experiment Station at Central Baraguá, and S. C. 12 (4) also proved to be rather susceptible, the spots being somewhat wider and longer than is the case on Cristalina and B H 10 (12).

Even though some experiments were under way at that time, it was still considered that this disease was probably immature eye spot. However, a serious outbreak of eye spot in Oriente Province, Cuba, in the winter of 1926–1927 indicated that these were two distinct Helminthosporium diseases on sugar cane. There was a marked difference in varietal susceptibility to the eye spot, the varieties D 109, FC 214, and FC 137 being so seriously attacked that the leaves were drying and in many cases the tops of the

plants were rotting, while in Cristalina fields alongside it was very difficult to find even the least trace of eye spot.

Four variety collections in this province, all of which came originally from Porto Rico, were then visited, and a heavy outbreak of eye spot was found in each of them. Cristalina cane growing alongside these heavily infected areas showed only the slightest traces of eye spot, and in one case none at all.

On the contrary, in two of our experimental plantings, one in Oriente Province and the other in Camagüey Province, we found the brown striping very plentiful on Cristalina as well as on many other varieties, but no eye spot was found. Close observation showed marked differences in the eye spot and brown stripe diseases, not only as to the susceptible varieties but also in the shape, form, and color of the spotting, and in the characters of the causative fungi, in average spore measurements and type of growth upon media.

Cook (7), in July, 1924, published a short note on leaf spot of sugar cane in which he thought he had two spot diseases due to *Helminthosporium*. One of these he termed the "Manati disease." The other he finds occurs only on B H 10 (12) cane at Guanica in Porto Rico. In October of the same year (6) he published a preliminary paper describing the *H. sacchari* in Porto Rico as being subject to "considerable variations which may be due to local conditions or to varieties of host plants or to unknown causes; that the Manati form is the same or a closely related species and the Santa Rita form is a variety or possibly a new species. However, it may be that the Santa Rita disease[1] as described may be due in part to other causes." As described, the spots of the Santa Rita disease fuse to form blotches of a red to dark purple color, the purple predominating and increasing to cover more than half the lower part of the leaf, while the "upper or outer half of the leaf shows very little or no spotting but with the advancement of the disease on the lower half it becomes yellow and ashy brown. The sheath is finally attacked but not until the disease is well advanced on the blade."

From this description we were certain that our brown stripe was not the same as this Santa Rita disease. In a recent paper, Cook (8) states that the two names given above have gone out of use and that the disease is now generally known as eye spot. He then states: "The first studies made by the writer showed considerable variations in the spots and in size of the spores which led us to suspect that the spotting might be due to two species of organism causing the disease but later studies have shown that this is not true."

[1] The disease on B H 10 (12), according to Faris.

GROWTH CHARACTERS OF THE FUNGI CAUSING BROWN STRIPE AND EYE SPOT

Parallel isolations and platings were made of both species of *Helminthosporium*, that causing the well known eye spot and that of the brown stripe, in order to make comparative studies.

Blocks of leaf tissue showing the typical brown stripes and eye spots were surface sterilized in 95 per cent alcohol, flamed and then plated in cornmeal agar (Shear's formula), cane juice agar (2 per cent cane juice), and 1 per cent sucrose agar. A very large percentage of such platings yielded pure cultures of the organism. From sporulating material of such cultures platings were made and single spore cultures were secured. Within a period of 24–48 hours these fungi sporulate freely in cornmeal agar. However, the growth characters of the colonies are more constant and distinctive on 1 per cent sucrose agar.

The growth of these two fungi is well illustrated in figures 1 and 2, which are photographs of cultures 18 days old on 1 per cent sucrose agar plates. Single spore cultures of these two fungi isolated and reisolated to

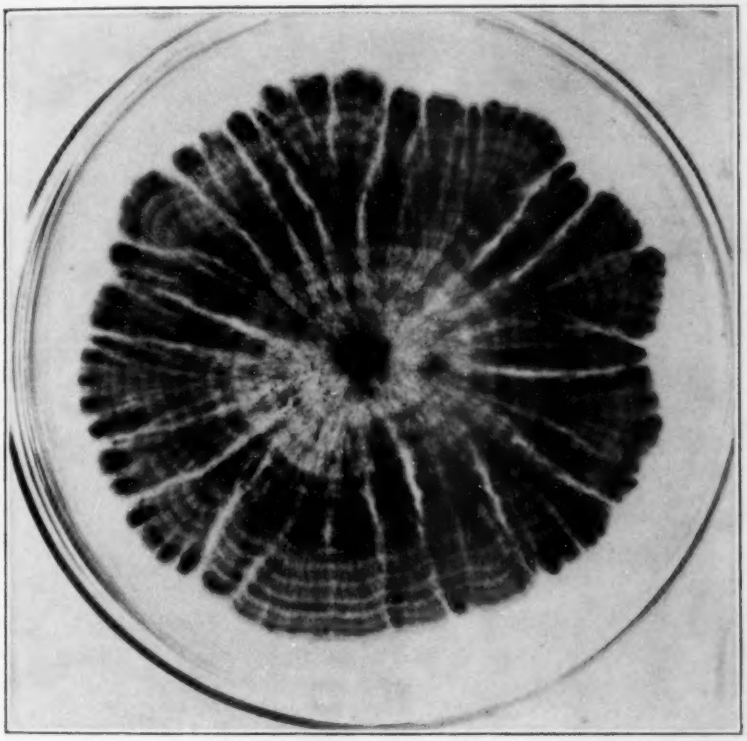


FIG. 1. Culture of eye spot *Helminthosporium* 18 days old on 1 per cent saccharose agar.

the fifth generation maintain these characteristic differences when grown on this medium and incubated at 25° C. On cornmeal agar and cane juice agar the growth characters were not so distinct.

The *Helminthosporium* causing brown stripe forms marked concentric rings, the hyphae growing radially from the spore or block of infected tissue, but lateral branches mingle and cross freely. The concentric rings formed are much broader than those of the eye spot fungus grown under the same conditions, and more nearly circular in outline. The concentric rings of the latter are rather irregular, owing to the type of growth which simulates dichotomous branching. Here again the growth is radial from the spore or block of infected tissue, but the hyphae soon divide and form sectors which seem to be repellent to one another and the lateral hyphae do not cross freely, thus leaving radial strips of agar between the sectors of fungous growth. This division continues into subdivisions, and a simulation of dichotomous branching is secured. These differences are clearly shown in figures 1 and 2.

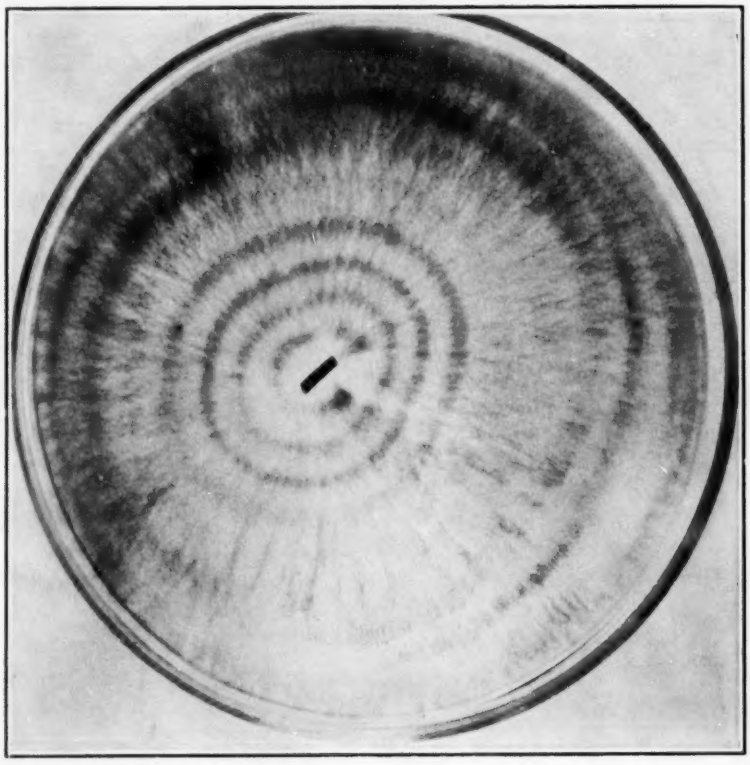


FIG. 2. Culture of brown stripe *Helminthosporium* 18 days old on 1 per cent saccharose agar.

While there are considerable differences in the growth of these two fungi on various media, when they are grown upon 1 per cent sucrose agar (2 per cent agar-agar) from single spore cultures, the broad concentric rings and free intermingling of lateral hyphae of the brown stripe organism are always in great contrast to the narrow concentric rings of broken outline, and sectorial growth of the eye spot *Helminthosporium*.

INFECTION EXPERIMENTS

The great profusion of brown stripe in the fields of Cristalina cane close to the laboratory made it impossible to use plants grown in the open for inoculation experiments. All plants used for studies in comparing the types of spots produced by the two Helminthosporiums in the early stages were germinated in chambers protected from outside spores. This was done in two ways. The simpler and less accurate method was to keep the pots in a chamber covered with a double layer of cheese-cloth and to make inoculations under a bell jar in this chamber.

This method was supplemented by the more accurate one of growing the plants under sterile conditions and making inoculations from pure single-spore cultures under aseptic conditions. Since this method presents some new features for the study of cane diseases, it is described somewhat in detail.

In glass cylinders 50 cms. high by 10 cms. in diameter was placed about 500 gms. of sand, moistened to about 70 per cent of its moisture-holding capacity with Schimper's normal culture solution. The cylinders were then stoppered with cotton, wrapped in paper, and sterilized 5 hours at 25 lbs. steam pressure in a large autoclave. At the same time smaller tubes holding 200 gms. of moistened sand were sterilized and the sand in these used to cover the cane when planted in the large cylinders.

Good seed pieces of the variety desired were thoroughly cleaned of all leaf parts, dirt, etc., and then immersed in water at 53.5° C. for 20 minutes, dipped in 95 per cent alcohol, soaked in 1–1000 $HgCl_2$ for 10 minutes and then planted without rinsing.

The cylinders were placed beneath a sterile hood and the paper wrapping removed. The seed cane was transferred from the mercuric bichloride solution into the sterilized glass cylinders under an aseptic hood, and a smaller tube of sterilized sand was used to cover the seed piece in the glass cylinders. In about 3 weeks the cane in these cylinders was ready for inoculation. A very high percentage of plants grown in this way were free from any contamination. In figure 3 the details of the apparatus described above are shown.

The jars were placed in the plant house for a period of 3 or 4 weeks until the plants had produced 5 or 6 leaves. They were then again transferred

to the asceptic hood and inoculated by means of a sterilized atomizer with single spore cultures of the *Helminthosporium* being studied. Great care was used to avoid contamination of any kind, and cultures which became contaminated were discarded.

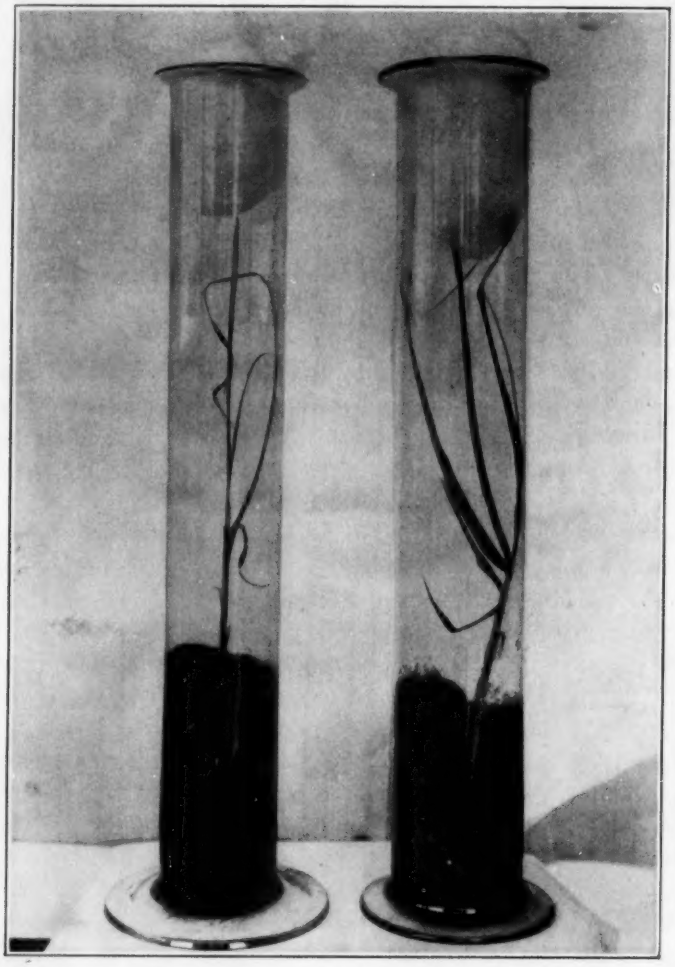


FIG. 3. Sugar cane plants growing under aseptic conditions.

By this method it was comparatively easy to demonstrate quickly and definitely the differences in the types of spots produced by the two species of *Helminthosporium*. The cultures under aseptic conditions were supplemented by pot cultures grown under cheese-cloth and inoculated by spray-

ing pure cultures of the organism and then placing bell jars over the plants for a period of three days.

It is believed that spores from diseased tissues produced under these aseptic conditions in a saturated atmosphere will be more comparable than when taken either from cultures, field material, or field collections placed in a moist chamber. That is, infected tissue killed by the parasite while attached to the plants, under conditions of saturated humidity and not in competition with other parasites, affords more nearly constant and comparable conditions than is secured by the methods usually employed in identifying and describing parasitic fungi.

Parallel experiments with single spore cultures of the fungi from eye spot and brown stripe were run.

Both types of spotting began to appear as very minute yellowish flecks within 48 hours. After 72 hours the spots were very numerous and had developed a reddish center. From this time on it was possible to distinguish between the two diseases on the F. C. 306 variety. The brown stripe became darker and elongated slowly to a definite linear stripe surrounded by a definite, yellowish green halo of fairly uniform width. This halo was, in general, about the width of the stripe and followed more or less the outline of the darker central portion, with perhaps a little greater width at the ends of the elongated spots.

The eye spot, on the other hand, did not turn so dark brown but remained of a brick-reddish cast. The spots were wider in relation to their length, but the most distinctive difference was the indefinite margins of the reddish central portion of the eye spot as compared with the definite margins of the dark brown central portion of the brown stripe, and the wider and more irregular halo of the eye spot. This wider halo is markedly longer in the lengthwise direction of the leaf, extending both ways from the longer axis of the spot, but soon develops long, lighter colored rays toward the tip of the leaf, which later become reddish to form the typical peacock-feather-like stripes as were described by van Breda de Haan (1). The brown stripe has never shown any tendency to extend in these long rays from the spots, even in the most susceptible varieties, such as Cristalina and B. H. 10 (12).

The differences described above are the same as those shown in the field by the two diseases. The plants grown in the jars are smaller and the spots are correspondingly smaller. The following description of the spots is made from field material of F. C. 306 and D 109, both of which are susceptible to both diseases (Table 4). The brown stripe material was taken from the plots of the Experiment Station at Baraguá, Cuba, where the eye spot does not exist at the present time. The eye spot material was taken from the experimental plots in western Camagüey Province upon plants badly diseased with eye spot but with very little brown stripe.

TABLE 4.—*Average sizes of 100 spots of brown stripe and eye spot on mature leaves of D 109 and F. C. 306 canes*

Cane variety	Disease	Total length in mm.		Width in mm.		Length-width ratio of spot
		Spot and halo	Spot	Spot	Halo	
F. C. 306	Brown stripe	25.0	22.9	1.16	1.05	20 to 1
	Eye spot	16.7	8.9	1.79	3.90	4.97 to 1
D 109	Brown stripe	21.4	19.5	1.00	0.95	20 to 1
	Eye spot	19.8	8.8	2.40	5.50	3.67 to 1

From table 4 it is clear that there is a very striking difference in the shape of the spots on these two varieties, the eye spot being about 1/4 as wide as it is long, while the brown stripe is about 1/20 as wide as long in typical cases. In the beginning stages these differences are not so great, but on mature leaves typical spots are about as above. The measurements of the width of the halo in the eye spot did not consider the long bands extending from the spots in advanced cases on these two varieties of cane. These bands are characteristic of the eye spot and have never been seen on any variety infected only with brown stripe. The width of halo with these two diseases varies with varieties, but so far as observed is usually greater in the case of the eye spot disease. A further marked difference is in the appearance of the actual spots themselves. The eye spot is not only of a brighter Bordeaux red color, but young spots have a stippled appearance rather than a solid color. Furthermore, the borders of the larger spots have this stippled appearance rather than the solid color of brown stripe. Also the halo surrounding the eye spot is from four to five times as wide as that around the brown stripe. These symptoms are so characteristic of the respective diseases that a little study in the field enables one to determine readily which disease is under observation.

The stripes, in the case of brown stripe, increase in size on many varieties, until they become 8–10 cm. long and up to ½ cm. in width. It is in these larger elongated spots that the fruits of the fungus appear in greatest abundance. The species causing brown stripe sporulates very sparsely as compared with the eye spot *Helminthosporium*, and it is with considerable difficulty that the spores are secured in any quantities from diseased leaves in the field. While sporulation may occur on the stripes of affected leaves in moist chambers, great care must be used to secure the maximum number of mature spores when measuring for identification. In the culture jars described, the brown stripes become thoroughly covered with the spores, and as the leaves die (without contamination in the aseptic

jars), the infected areas become densely covered with conidiophores and spores. The measurements taken for both the eye spot and brown stripe

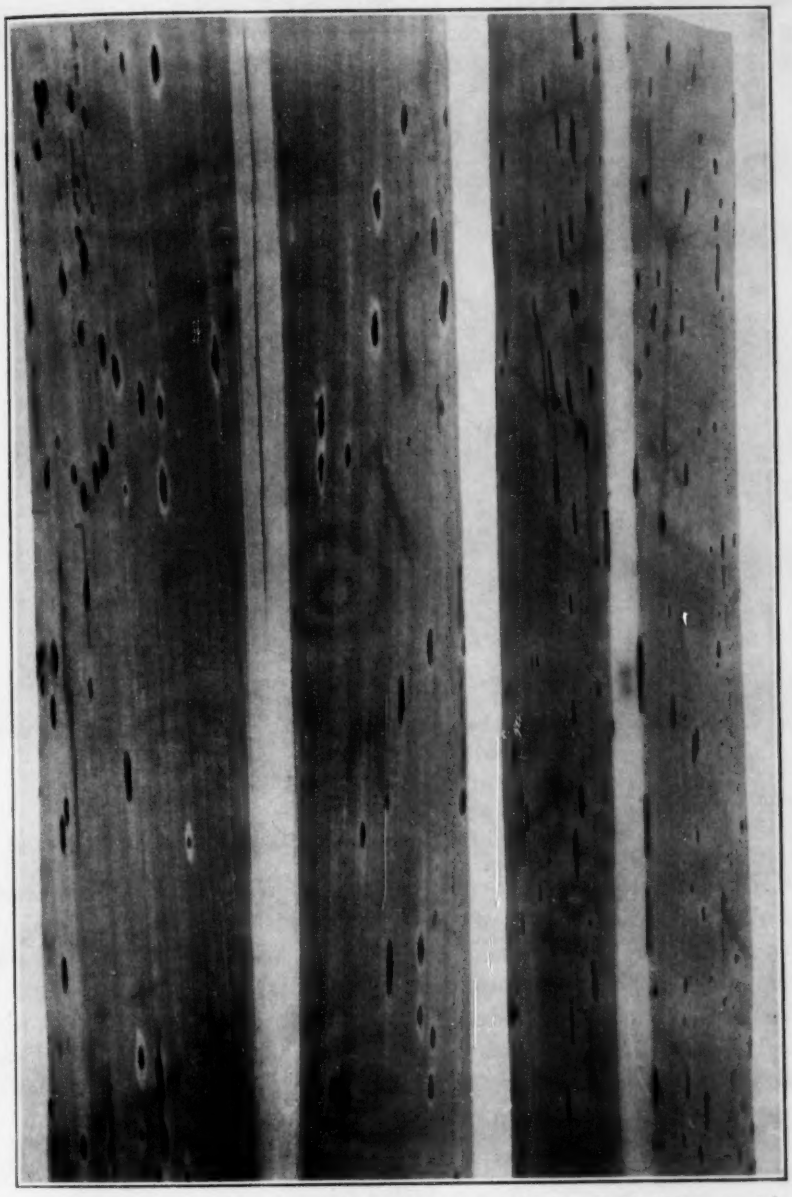


FIG. 4. Left—Typical eye spot on D 109 cane leaf. Right—Typical brown stripe on Cristalina cane leaf.

species of *Helminthosporium* were secured from material taken from these culture jars. Contrary to results secured from field collections the average measurements of spores from the jars were remarkably uniform, varying less than 3 μ in length from the general average given and less than ½ μ in width when several lots of 100 or more spores were measured.

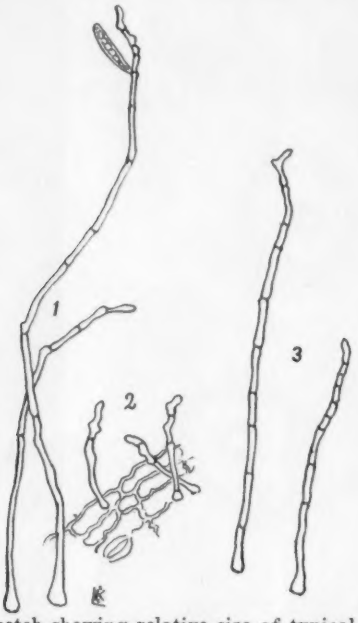


FIG. 5. Camera lucida sketch showing relative size of typical conidiophores of three cane *Helminthosporiums*.

1—Conidiophores of *Helminthosporium ocellum* n. sp.
2—Conidiophores of *H. sacchari* Butler.
3—Conidiophores of *H. stenospilum* Drechsler.

Under such conditions the spores of the *Helminthosporium* causing brown stripe are of a dark olivaceous color with a thick peripheral wall which is considerably thickened on the convex side. In my measurements of more than 1,000 spores produced in the glass cylinders previously described, the range of spore length was from 54 to 131.6 μ and of spore width from 11.3 to 18.8 μ with weighted averages of 89.6 μ and 15 μ respectively. These measurements are slightly greater than those given by Drechsler (9) for the brown stripe organism, which he has named *H. stenospilum*.

Varietal Susceptibility of Sugar Cane to the Brown Stripe Disease

Table 5 indicates the susceptibility of the principal cane varieties to this disease when grown under field conditions at Central Baraguá, Cuba.

TABLE 5.—*Relative susceptibility of cane varieties to brown stripe in Cuba*

GROUP I.—Varieties which are very susceptible. The new leaves become thoroughly covered with stripes and elongated spots.

Cristalina	B H 10 (12)
Caña Blanca	Morada (La. Purple)
B 3696	P R 16/6
B 3405	POJ 2221

GROUP II.—Varieties less susceptible than Group I.

Badila	C 291
S C 12/4	C 483
B 1809	D 109
B 6450	D-1135
P R 719	F C 306
D 216	Kassoer
C 555	B 3412
D 74	D 116
D 117	

GROUP III.—Varieties with moderate infection upon the older leaves.

Java Unknown	P R 712
H 109	P R 724
FC 137	B 4596
FC 214	Caña Criolla
POJ 2725	Negrita
P R 16/13	C 35
P R 209	POJ 36
P R 701	B 208
P R 704	C 519
P R 707	POJ 2714
P R 709	Bambo Blanca
B 109	Bambo Morada
C 291	C 553
Yellow Caledonia	D 99
	D 108

GROUP IV.—Varieties slightly susceptible or resistant.

Kavangire	Cayana 10	T 507
D 433	M 36	Uba
C 390	POJ 234	Cavangire
Tjepering	POJ 826	C 64/21
Co 210	POJ 979	C 653
Co 213	POJ 2379	Penang
Co 214	POJ 2727	
Co 232	Burra	
Co 281	SPI 33243	
POJ 228	Merthi	
	Oshima	
	Teckcha	
	T 439	

Field Losses from Brown Stripe

Brown stripe, while it would not be considered a major cane disease, as is the eye spot, is one of the most destructive of the minor cane diseases in Cuba. It becomes destructive during periods of dry weather when the vitality of the cane is lowered, and at times the stripes are so profuse as thoroughly to cover the leaves of the more susceptible varieties. Under such conditions the disease hastens the death and drying of the cane leaves. Vigorously growing cane suffers slight injury, and the only control measures justified at present are such cultural operations as may economically be carried out to maintain vigorous growth of the plants.

SUMMARY

1. The eye spot disease of sugar cane is widely distributed in Cuba, where it is destructive upon certain varieties of cane.

2. This disease, previously attributed to *Helminthosporium sacchari* Butl., does not agree in symptoms with the Helminthosporiose described and figured by Butler and Hafiz.

3. The organism causing eye spot is a *Helminthosporium* but differs from *H. sacchari* Butler and *H. stenospilum* Dreschler in color and length of spores and sporophores, as well as in the type of lesions produced upon the host plant.

4. The eye spot organism is provisionally named *Helminthosporium ocellum*.

5. A third Helminthosporium disease of sugar cane, brown stripe, has been found. Proof of parasitism of the causal organism, *H. stenospilum*, has been made by pot cultures and inoculation of plants growing under aseptic conditions.

6. A method for growing and inoculating plants under aseptic conditions has been worked out, which, it is believed, will enable a comparison of parasitic fungi under much more uniform conditions than is secured from field collections or the usual moist chamber methods.

7. Cane varieties differ greatly in their susceptibility to the brown stripe and eye spot Helminthosporiums, both of which are widely distributed in Cuba.

TROPICAL PLANT RESEARCH FOUNDATION

LITERATURE CITED

1. BREDA DE HAAN, J. VAN. Oogvlekken-ziekte door *Cercospora sacchari* n. sp. Meded. Proefsta. Suikerriet West-Java **3**: 15–21. 1892.
2. BRUNER, S. C. Lista preliminar de las enfermedades de las plantas de importancia económica para Cuba. Cuba Estac. Exp. Agron. Informe **1918–20**: 723–775. (See pp. 735, 739.) 1920.

3. BUTLER, E. J. Fungi and disease in plants. 547 pp. Calcutta and Simla. (See pp. 406–408, figs. 162 and 169.) 1918.
4. ———— and A. HAFIZ KHAN. Some new sugarcane diseases. India Dept. Agr. Mem., Bot. Ser., 6[4]: 181–208. (See Pl. I, fig. 3, and Pl. VI, figs. 1–8.) 1913.
5. COBB, N. A. Fungus maladies of the sugar cane. Hawaiian Sugar Planters' Exp. Sta. Bul. 6. (See Pl. IV.) 1909.
6. COOK, M. T. Helminthosporium leaf spot of sugar cane in Porto Rico. (Preliminary paper.) Jour. Dept. Agr. Porto Rico 8[4]: 5–10. 1924.
7. ————. Informe annual de la division de patologia vegetal y botanica para el año fiscal de 1923–24. Puerto Rico Estac. Exp. Insular. Informe An. 1923–24: 89–100. (See p. 81.) 1924.
8. ————. The eye-spot disease of sugar cane. Jour. Dept. Agr. Porto Rico (1926) 10: 207–227. 1927.
9. DRECHSLER, C. A species of *Helminthosporium* distinct from *Helminthosporium sacchari*, causing brown stripe of sugar cane (Abst.). Phytopath. 18: 135–136. 1928.
10. FARIS, J. A. Some sugar cane leaf spots in Cuba. Facts About Sugar 22: 1183–1184. 1927.
11. ————. Brown stripe of sugar cane in Cuba (Abst.). Phytopath. 18: 135. 1928.
12. ————. Sugar cane eye spot in Cuba (Abst.). Phytopath. 18: 135. 1928.
13. JOHNSTON, J. R., and J. A. STEVENSON. Sugar-cane fungi and diseases of Porto Rico. Jour. Dept. Agr. Porto Rico 1: 177–251. 1917.
14. ————, S. F. ASHBY, C. K. BANCROFT, W. NOWELL, and J. H. STEVENSON. Diseases of sugar-cane in tropical and subtropical America, especially the West Indies. West Indian Bul. 16: 275–308. 1918.
15. KRÜGER, W. Das Zuckerrohr und seine Kultur. 580 pp. Magdeburg and Wien. (See pp. 450–453, Tafel. XI.) 1899.
16. LEE, H. A. The history and distribution of eye spot. Hawaiian Planters' Rec. 30: 466–470. 1926.
17. ————. Losses caused by eye spot. Hawaiian Planters' Rec. 30: 472–474. 1926.
18. ———— and J. P. MARTIN. Description of eye spot as compared with other sugar cane leaf spot diseases. Hawaiian Planters' Rec. 30: 470–471. 1926.
19. ————, ————, and C. C. BARNUM. A method of testing cane varieties for eye spot susceptibility and resistance. Hawaiian Planters' Rec. 30: 487–492. 1926.
20. LUCASSEN, T., and F. A. F. C. WENT. Afbeeldingen van Rietziekten. Meded. Proefsta. Suikerriet West-Java 16. 7 pp. (See Pl. III.) 1894.
21. MARTIN, J. P., and H. K. STENDER. The control of eye spot through resistant varieties. Hawaiian Planters' Rec. 30: 484–487. 1926.
22. WAKKER, J. H., and F. A. F. C. WENT. Overzicht van de Ziekten van het suikerriet op Java. Meded. Proefsta. Oost-Java (N. S.) 22: 11 pp. (Meded. Proefsta. Suikerriet West-Java 24.) (See p. 7.) 1896.
23. ———— and ————. De Ziekten van het Suikerriet op Java. Deel 1. Leiden. (See pp. 157–162, Pl. XXI, figs. 1–5.) 1898.

EXPLANATION OF PLATE XV

I. Early stage of brown stripe disease upon Cristalina cane.

II. Brown stripe in a late stage of the disease upon Cristalina cane.

III. Early stage of eye spot upon D 109 cane.

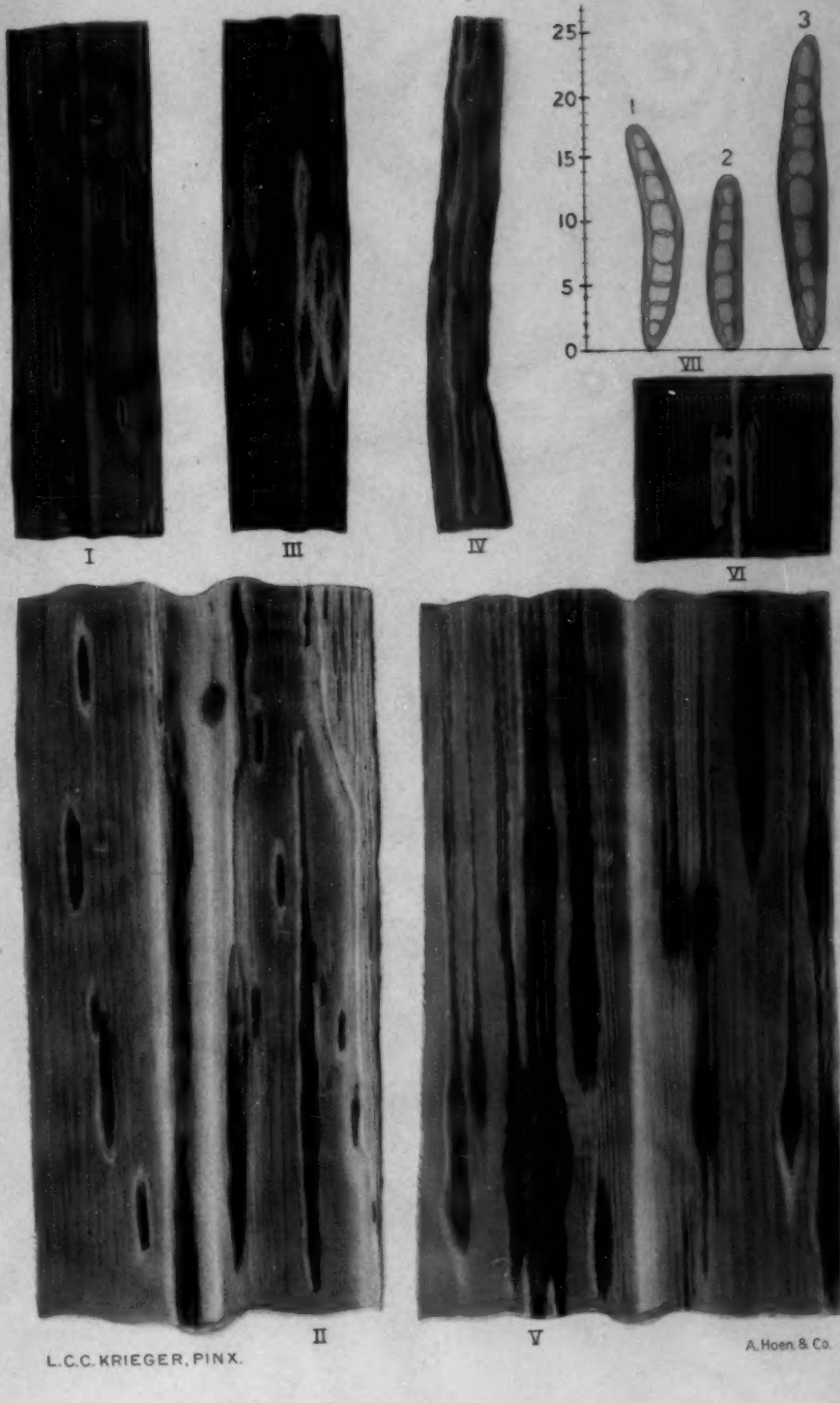
IV. Late stage of eye spot disease upon young B 3412 cane. The original spots enlarge to form a blotch.

V. Eye spot in a late stage of the disease upon D 109 cane.

VI. Copy of helminthosporiose of cane leaf—as shown by Butler and Hafiz on their Plate I, fig. 3 (4).

VII. Typical spores of average size of three parasitic Helminthosporium species on cane drawn to the same scale.

1. Spore of *Helminthosporium ocellum* n. sp., causing the eye spot disease of cane.
2. Spore of *Helminthosporium sacchari* Butler, causing the Helminthosporiose disease of cane.
3. Spore of *Helminthosporium stenospilum* Drechsler, causing the brown stripe disease of cane.



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THE EFFECT OF A MANGANESE DEFICIENCY ON THE SUGAR CANE PLANT AND ITS RELATIONSHIP TO PAHALA BLIGHT OF SUGAR CANE

H. Atherton Lee and J. S. McHargue

INTRODUCTION

Pahala blight is a disease of sugar cane which takes its name from the locality where it was first observed and most commonly occurs, Pahala, a small town towards the southern extremity of the island of Hawaii, of the Hawaiian group. There is very little literature concerning this disease; Cobb (1) first described the disease in 1906, ascribing it to the leaf-splitting fungus, *Mycosphaerella striatiformans*. Cobb (2) also published fine colored illustrations of the leaf symptoms of the disease in 1909.

Since then the disease has been observed recently at Olaa on the island of Hawaii, and at Kilauea on the island of Kauai; but other than these instances in the Hawaiian Islands, there is no knowledge of the occurrence of this disease of sugar cane in other countries.

DESCRIPTION OF PAHALA BLIGHT

Pahala blight is not well named from the pathologist's viewpoint, for the disease is not a blight but is more properly a partial chlorosis: the leaves lose their chlorophyll in long, white streaks. There are many different degrees of severity of Pahala blight symptoms, but the most commonly occurring symptoms are the presence on the leaves of these long, whitish chlorotic streaks which are confined to the leaf blades and do not extend down into the leaf sheaths (Fig. 1). It is usually the third, fourth and fifth leaves from the youngest unfolded leaf of the cane top which show the symptoms most noticeably. These streaks may occupy different proportions of the leaf area, depending on the severity of the disease. As the disease continues, small reddish spots occur along the length of the white streaks, due to the killing of the white tissues. On these red spots the fungus *Mycosphaerella striatiformans*, found by Cobb and at the time thought to be the cause of the disease, is frequently found. Plants affected to such a degree that red spots begin to appear are usually considerably stunted by the disease. The red spots may become so numerous as to cause continuous reddish-brown streaks. In advanced cases the leaves of blight-

affected plants split along the reddened streaks (Fig. 2), and at one time blight was known to some extent as the leaf-splitting disease. Pahala blight of sugar cane in our experience has always occurred in soils of a neutral or alkaline reaction.

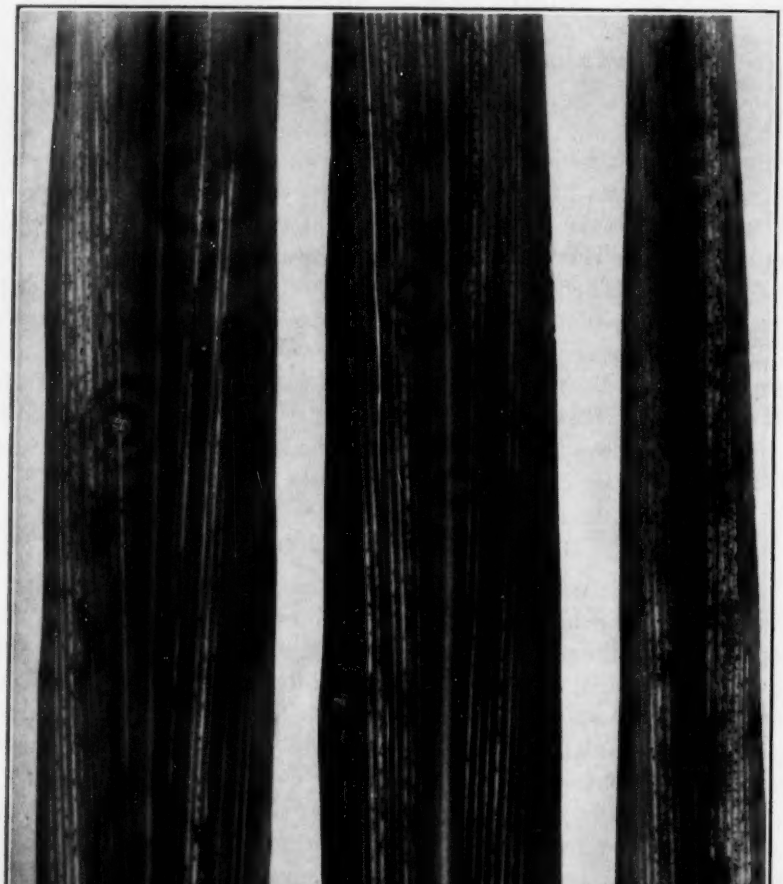

FIG. 1. Pahala blight symptoms on leaves of Badila variety of sugar cane, showing chlorotic streaks with red spots beginning to appear.

DISEASES WITH WHICH PAHALA BLIGHT MIGHT BE CONFUSED

In sugar cane there are leaf mutations with white streaks sometimes called sports, similar to those found in corn, which by one unfamiliar with these mutations might be confused with blight. However, in the case of such white leaf sports, the edges of the streaks are very definite and clearly defined; moreover, in the case of these sports there are usually but one or two streaks, at most five or six, while in the case of Pahala blight the longitudinal streaks are usually narrower but much more frequent on a single leaf. A sport, moreover, may usually be identified by finding the white streaks in a definite given position on succeeding leaves on the same side of the cane stalk, a distribution which would not apply to the streaks of Pahala blight.

Leaf scald, a disease of sugar cane in Australia, Fiji, Java, and the Philippines, in its early stages also causes a white longitudinal streak. Such a streak frequently runs down on to the leaf sheath, which is a quite distinct character from the chlorotic streaks of Pahala blight which are mostly confined to the leaf blades; the streaks of leaf scald are also wider, and not so numerous on a single leaf. The leaf streak disease of sugar cane in Natal, Egypt, and Mauritius might be confusing in the literature but should be easily distinguished by its yellowish streaks rather than the chlorotic whitish streaks typical of blight.

Pahala blight might also be confused with limestone chlorosis and other forms of chlorosis in which iron is deficient. In these iron-deficiency forms of chlorosis the whole leaf turns a whitish or whitish yellow color; in such types of chlorosis the streaking is not so definite as in the case of Pahala blight, and there is usually not the stunting effect on the plant as in the case of Pahala blight.

PREVIOUS EXPERIMENTS ON PAHALA BLIGHT

Some experiments with Pahala blight of sugar cane were described by Williams (7) in 1920, in which he was able to avoid the disease in a field in which blight usually occurred by (*1*) excavating a plot from the affected field and substituting a volcanic mud-flow soil from a field in which blighted plants had never been found; and (*2*) incorporating a large proportion of organic matter (stable manure mixed with sugar-cane press cake and bagasse) in plots with the usual field soil. In plots where a layer of charcoal was placed under the usual field soil, for the purpose of absorbing possible toxic gases arising through the soil, the disease reoccurred without abatement. Williams concluded (*1*) that the cause of Pahala blight resided in the soil, (*2*) was of a character other than toxic gases, and (*3*) could be corrected by adding organic matter to the soil.

FIG. 2. Pahala blight symptoms on leaves of Badila variety of sugar cane, showing red streaks following chlorotic streaks, and the splitting of the leaves along such streaks.

In 1925 McGeorge (3) showed both experimentally and in field tests that applications of sulphur to the soil at the rate of from 1 to 2 tons an acre resulted in an entire recovery of the cane from Pahala blight in from 4 to 6 months after the treatment. He concluded that Pahala blight is a physiological or nutritional disturbance induced by soil conditions. McGeorge presented some analyses of blighted leaves as contrasted with normal leaves and it is interesting to note that there is no correlation between the presence or absence of iron in the leaves and the presence or absence of blight.

FIELD EXPERIMENTS WITH MANGANESE

McLean and Gilbert (5), in Rhode Island, had just shown that manganese solutions applied to the foliage of chlorotic spinach would bring about a quick recovery from the chlorosis, and the senior author (Lee), following their work, was experimenting with iron and manganese in relation to Pahala blight. Manganous sulphate and iron (ferrous) sulphate in solutions and as dry dusts were applied to the cane foliage of plants affected with the blight. The manganous sulphate brought about a prompt recovery of the cane, while the iron sulphate burned the leaves. This led to a more complete experiment in which manganous sulphate crystals were powdered and mixed with fungicidal dusting sulphur, the latter serving merely as a carrier, and applied to the foliage. Three different combinations of manganous sulphate, in sulphur as a carrier, were tried as contrasted with iron sulphate also in a sulphur carrier: there were four replications of the plots of each treatment, each plot consisting of four rows of cane, each row about 60 feet long. The manganous sulphate and iron sulphate crystals were not dehydrated but were merely crushed and ground fine before being added to and mixed with the sulphur carriers. The response to the successful treatments was noticeable five days after application and increased to a very considerable degree of recovery in three weeks. A description of the results of these treatments is contained in a letter of November 4, 1925, from Mr. J. C. Thompson, Research Agriculturist for the Hawaiian Agricultural Company, at Pahala, Hawaii, which is quoted as follows.

"In view of your request for a report on your dust experiments in Fields Middle Naahala and Lower Aliona, put in on October 16 in connection with the Pahala blight investigation, I beg herewith to report as follows:

First Series of Replications

Check Plot: Not very badly blighted, but blight found evenly distributed through the four lines of cane in the plot.

Dust No. 13:

20% Iron sulphate: Apparently no result from present appearances; dusted plot same as check plot.

Dust No. 14:

20% Manganous sulphate: Cane almost fully recovered. Good dark green color as compared to light green of check plot. Manganous sulphate action on blighted leaves found, that is, the characteristic well pronounced dark green-colored stripes and the yellowish white diseased stripes.

Dust No. 15:

30% Manganous sulphate: Practically identical results as with Dust No. 14.

Dust No. 16:

20% Manganous sulphate plus 15% ammonium sulphate: Almost fully recovered with the stand a dark green color as compared to the lighter green check plot.

Second Series of Replications

Check Plot: Cane stand consisting of four lines of cane very badly blighted and all yellow.

Dust No. 13:

20% Iron sulphate: No apparent difference between dusted plot and check plot.

Dust No. 14:

20% Manganous sulphate: Fair recovery although not so pronounced as to be noticed immediately. Manganous sulphate characteristic throughout when compared with check plot.

Dust No. 15:

30% Manganous sulphate: A better recovery than with Dust No. 14.

Dust No. 16:

Manganous sulphate, 20%; ammonium sulphate, 15%: Best recovery as far as this series is concerned, although not very pronounced. Sufficient difference between this plot and check plot, but no total recovery.

Third Series of Replications

Check Plot: Four lines blighted throughout.

Dust No. 13:

20% Iron sulphate: No apparent difference between this plot and check plot.

Dust No. 14:

20% Manganous sulphate: Fairly good green stand. Some blighted leaves but fairly dark green throughout as compared to the yellow blighty stand of the check plot.

Dust No. 15:

30% Manganous sulphate: Almost total recovery from blight. Good dark green healthy color except for the lighter-green mosaic leaves. Check plot light yellow and blighty color.

Dust No. 16:

Manganous sulphate, 20%; ammonium sulphate, 15%: Dark green and almost total recovery of shoots except for mosaic and a few blighted leaves with characteristic manganous sulphate tinge. Check plot yellow and blighted.

Fourth Series of Replications

Check Plot: Fairly good stand although it still retains its yellow tinge.

Dust No. 13:

20% Iron sulphate: No apparent action on blight. Leaves of whole plot badly burnt, more especially in case of diseased leaves. Healthy leaves also burnt.

Dust No. 14:

20% Manganous sulphate: Dark green stand good recovery with slight blight left. Manganous characteristic on leaves. New leaves healthy. Same for both plots; check plots light green with blighty tinge.

Dust No. 15:

30% Manganous sulphate:

(a) Fair recovery but not so noticeable due to mosaic disease in the plot which gives a rather light greenish color. Poor stand in this plot. Manganous sulphate characteristic of leaves present. Check plot fairly blighted.

(b) This plot showed fairly good recovery with fairly good green color to leaves. Few blighted leaves. Slight amount of mosaic disease present. Check plot only slightly blighted, with mosaic present also.

Dust No. 16:

20% Manganous sulphate; 15% ammonium sulphate: Good recovery. Some wholly recovered. New leaves healthy. Dark green color characteristic of manganese-treated leaves very pronounced. Check plot, yellow and blighty."

To summarize the results: iron sulphate resulted in no indications of recovery by the cane; on the contrary, iron sulphate resulted in a burning of the foliage of cane plants affected by blight. On the other hand manganous sulphate in a 20 per cent mixture in sulphur brought about a considerable recovery of the cane from blight. The mixture of 30 per cent manganous sulphate in sulphur seemed to allow plants a better chance of recovery than the 20 per cent mixture. The mixture of 20 per cent manganous sulphate and 10 per cent ammonium sulphate in sulphur seemed to induce better recovery than any of the other mixtures.

In conversation, P. S. Burgess, now of the Arizona Agricultural Experiment Station and formerly of the Experiment Station of the Hawaiian Sugar Planters' Association in Honolulu, stated that he tried out iron sulphate treatments against Pahala blight of sugar cane, with negative results similar to those just recorded.

Although it is a common impression that mineral elements are not absorbed through the cuticle by the leaves of plants, yet there has been at times some evidence in California (Ballard) that nitrogen from nitrate of soda was absorbed by the leaves of apple trees when applied as liquid spray. Certainly the present results indicate that manganese and possibly nitrogen from ammonium sulphate are absorbed and utilized by the leaves when applied to the foliage of sugar cane.

Subsequent to these results at Pahala, a considerable recovery from Pahala blight at the Kilauea Sugar Company was secured with applications of 30 per cent powdered manganous sulphate crystals in a sulphur carrier as shown in a letter of August 19, 1926, from Mr. Royden Bryan, Assistant Agriculturist at the Experiment Station of the Hawaiian Sugar Planters' Association, Kilauea, Kauai.

"Herewith submitted is a report of the effect of manganous sulphate against Pahala blight in Field No. 28, Kilauea Sugar Plantation Company.

The cane is the variety U. D. 1, ratoons, unirrigated. The plots, two treated, and two untreated as checks, each consists of 4 rows of cane; each row about 80 feet long. The treated plots were dusted with 30 per cent manganous sulphate in a sulphur carrier on July 2, 1926. Upon visiting the experiment on August 17, 1926, 45 days later, I observed a vast improvement in the treated plots. Although the cane in the checks showed a slight recovery from the blight, that in the treated plots had almost fully recovered, being ahead in growth and the new leaves free from Pahala blight.

Conclusion: U. D. 1 cane having Pahala blight showed an almost complete recovery from the blight, 45 days after being dusted with manganous sulphate."

From these field results, both at Pahala on the island of Hawaii and at Kilauea on the island of Kauai, there was a strong suggestion of relationship between the presence of manganese and the occurrence of Pahala blight. As stated previously, the occurrence of blight in the Hawaiian Islands has in all cases been in neutral or slightly alkaline soils. The working theory advanced was that sulphur applications to the soil, found by McGeorge to be so successful in curing Pahala blight, brought about a change in the soil reaction from neutral or slightly alkaline to acid and that in an acid soil complex, manganese was available to the plants, while in the original neutral or alkaline soil complex, manganese was not sufficiently available to the plants.

Experiments were therefore undertaken by the junior author (McHargue) to prove or disprove this theory that a manganese deficiency is the cause of Pahala blight of sugar cane.

CHEMICAL ANALYSES OF PAHALA BLIGHT LEAVES

First the presence or absence of iron and manganese in normal, partially affected, and severely affected Pahala blight plants was determined by analysis. The results of these analyses are shown in table 1.

These analyses showed that whereas there was no correlation between the iron content of leaves and the severity of Pahala blight, there was a direct correlation between the manganese content of such leaves and Pahala blight.

Previous work on manganese as a plant constituent had failed to show its essential relationship to plant growth. The junior author of this paper

[McHargue (4)], however, showed that many chemical reagents have manganese impurities, a factor which was not recognized in earlier experiments, and such impurities in supposedly manganese-free culture solutions nullified the value of the experiments. He showed in experiments with carefully purified chemicals in sand and solution cultures that manganese was, and is, an essential constituent of many plants.

TABLE 1.—*Analyses of normal, semi-chlorotic, and chlorotic sugar cane leaves*

Material present	Normal green leaves Per cent	Semi-chlorotic leaves Per cent	Chlorotic leaves Per cent
Ash (crude)	4.236	3.663	4.553
Iron (Fe)	0.036	0.016	0.024
Manganese (Mn)	0.003	0.0005	trace
Calcium (Ca)	0.224	0.333	0.380
Magnesium	0.232	0.146	0.154
Phosphorus (P)	0.155	0.173	0.220
Potassium (K)	1.455	1.441	1.648
Sodium (Na)	0.425	0.580	0.562
Sulphur (S)	0.161	0.267	0.240
Nitrogen (N)	1.305	1.650	1.690
Protein (N. × 625)	8.156	10.313	10.560

In this sand culture work the symptoms of manganese deficiency in such plants as oats and wheat were strongly suggestive of the symptoms of Pahala blight of sugar cane. This led to the present experiments in which young plants of sugar cane were grown in manganese-free solutions to determine whether such an absence of manganese would cause the Pahala blight symptoms.

Vegetative cane cuttings of the Yellow Caledonia variety, a variety which is very susceptible to Pahala blight, were secured from the United States Department of Agriculture, Sugar Cane Breeding Station, at Canal Point, Florida, through the cooperation of Messrs. E. W. Brandes, R. D. Rands, and B. A. Bourne. These cuttings were planted in manganese-free sand cultures, germinated, and grown to a point where the cane shoots formed from the cutting had sufficient roots to be independent of the cane cuttings. The cuttings were then dug up and separated from the growing shoots with a knife; the growing shoots with their own roots were then planted in new manganese-free sand cultures. There were ten of such manganese-free cultures. The purpose of separating these cane cuttings was to eliminate any sources of manganese in the cuttings which might be available to the growing shoot and which would thus spoil the experiment with manganese-free cultures.

Ten cuttings were planted in sand cultures identical to the preceding except that manganese was included in the culture solution; the seed-piece cuttings were excised from the growing shoots in the same way and at the same time as the plants which were grown in the manganese-free cultures. All plants were then maintained under identical environmental conditions until the conclusion of the experiment.

The experiment was started in early September, 1926, in greenhouses at Lexington, Kentucky. The plants germinated, grew well, and were transplanted successfully, but as the winter months approached the plants did not grow in the greenhouse so well and vigorously as would have been the case in the open.

By February, 1927, all plants in the manganese-free cultures were showing the typical chlorotic leaf streaks and stunted appearance of Pahala blight. The control plants, with manganese present, under identical environmental conditions, grew a normal, luxuriant green foliage.

The experiment appears to prove rather definitely that Pahala blight of sugar cane is due to a deficiency of manganese in the available food supplies of the plant. The conclusions from these results with manganese-free sand cultures are supported by the recovery of sugar cane from Pahala blight when manganese was applied to the foliage.

DISCUSSION

A presentation of the subject would be incomplete if mention were not made of the results obtained by Schreiner and Dawson (6); they showed that a chlorosis of tomatoes occurring in Florida, on neutral or alkaline soils, was completely cured by additions of manganese sulphate to the soil. A recovery from the disease was also secured by adding stable manure to the soil. The leaf symptoms of their tomato chlorosis were identical with the Pahala blight symptoms of sugar cane. Apparently the two diseases are identical in nature.

The action of sulphur on the soil to increase the soil H-ion concentration and so make manganese in the soil complex more readily available to the plant, is in support of the experimental results recorded here.

There is also a relation of this nutritional disturbance to the use of ordinary fertilizer materials. A physiologically alkaline salt such as sodium nitrate, when used as a source of nitrogen, would considerably accentuate the disease, while the physiologically acid salt, ammonium sulphate, in itself, has been shown by plantation experience at Pahala to minimize the blight considerably. Acid superphosphate, which undoubtedly will have manganese impurities and would also be expected to have a slight tendency to increase the H-ion concentration of the soil, also has a slightly beneficial effect on Pahala blight in field practice.

Additions of organic matter to the soil probably alleviate Pahala blight not only by introducing small quantities of available manganese into the soil, but also by their action in increasing the soil H-ion concentration slightly and thus making some of the soil manganese more readily available.

It is apparent to the present writers that there are considerable differences among sugar cane varieties with respect to their demand for manganese. Yellow Caledonia, Yellow Bamboo, and possibly Badila, which are cane varieties very susceptible to Pahala blight, we would regard as active manganese feeders. On the other hand, resistant varieties such as D-1135 and possibly H-109 seem to have considerably less demand for manganese.

Since the name Pahala blight is not very appropriate from the viewpoint of plant pathologists, the more informative names, manganese deficiency, or manganese-deficiency chlorosis, are suggested as more accurate designations for this disease.

To the writers, one of the most interesting observations in connection with this work has been that what seemed abstract, or pure, research results obtained in one part of the world, *i.e.*, Kentucky, were so quickly utilized in an economic problem in Hawaii, in a rather distant part of the world. The writers feel that the results are also an outstanding evidence of the great value of cooperation.

SUMMARY

1. Applications of manganous sulphate to the leaves of sugar cane affected with Pahala blight brought about an alleviation of the disease and subsequent increased growth.

2. On analysis, leaves of sugar cane with severe Pahala blight symptoms showed only traces of manganese; leaves with slight symptoms of blight showed only slightly more manganese, while leaves of normal cane showed several times as much manganese. There is thus a correlation between the manganese content of leaves and Pahala blight.

3. There was no correlation between the iron content of sugar cane leaves and the presence or absence of Pahala blight symptoms.

4. Sugar cane plants grown in manganese-free sand cultures gradually developed Pahala blight symptoms until, at the end of six months, the disease was quite severe. Identical plants, except that they had adequate manganese, under identical environmental conditions, grew normally. From the results of this experiment the conclusion seems evident that Pahala blight results from a manganese deficiency. These experimental results and

conclusions are supported by the analytical results and the favorable response of the cane plant to manganese applications in the field.

EXPERIMENT STATION HAWAIIAN SUGAR PLANTERS' ASSOCIATION
AND
KENTUCKY AGRICULTURAL EXPERIMENT STATION.

LITERATURE CITED

1. COBB, N. A. Fungus maladies of the cane. Div. of Pathology and Physiology, Exp. Sta. Hawaiian Sugar Planters' Assoc. Bul. 5: 93. 1906.
2. ———. Fungus maladies of the cane. Div. of Pathology and Physiology, Exp. Sta. Hawaiian Sugar Planters' Assoc. Bul. 6: Plate VII. 1909.
3. MCGEORGE, W. T. Pahala blight and a comparison with other forms of sugar cane chlorosis. Hawaiian Planters' Record. 30[2]: 293. 1926.
4. MCHARGUE, J. S. Manganese and plant growth. Industrial and Engineering Chemistry, 18: 172. 1926.
5. MCLEAN, FORMAN T., and BASIL E. GILBERT. Manganese as a cure for chlorosis of spinach. Science 61: 636. 1925.
6. SCHREINER, OSWALD, and PAUL R. DAWSON. Manganese deficiency in soils and fertilizers. Industrial and Engineering Chemistry 19: 400. 1927.
7. WILLIAMS, W. L. S. Pahala blight investigations. Hawaiian Planters' Record 13[4]: 199. 1920.

POWDERY MILDEW OF RASPBERRY

P. D. Peterson and H. W. Johnson

INTRODUCTION

Powdery mildew of raspberry was not reported in Minnesota until 1923 although it probably had been present prior to that time. It was common on both red and black varieties in that year (3). Since 1923 the disease has become more and more abundant, assuming epidemic proportions in plantings of the Latham variety throughout the state in 1925, 1926, and 1927. Damage, as indicated by stunting of the canes, has been confined to this variety of red raspberry. Local leaf infections of mildew have been found on other varieties both of red and black raspberries, also on blackberry and dewberry, but such infections are never abundant. The perithecial stage of the mildew has not been found in Minnesota on any of the varieties of raspberry and blackberry which are attacked.

A survey of the literature leads one to believe that the powdery mildew is not confined to Minnesota. A similar—perhaps the same—mildew has been reported on raspberries in Ohio, New York, Washington, Connecticut, Illinois, Maryland, Oregon, and Indiana. In 1920 an undetermined powdery mildew was reported on raspberries in Ohio (5). In 1922 Rankin (6) reported a similar powdery mildew in New York as the cause of cane dwarfing in several varieties of red and purple raspberries. He again called attention to this mildew in 1924 (7), stating that the Latham and Owasco varieties were most susceptible. He also observed infection on varieties of black raspberry. In 1926 Zeller (8) reported powdery mildew as the cause of severe injury to the Munger variety in Oregon. In 1927 the senior author observed powdery mildew on the Latham variety in Michigan and Wisconsin. The identity of the mildew has not been determined in any of these states. The ease with which the organism might be distributed on nursery stock, a point which will be brought out later in this paper, suggests the possibility that the mildew in most of these areas is the same as the mildew in Minnesota.

THE CAUSAL ORGANISM—ITS POSSIBLE IDENTITY

The occurrence of *Sphaerotheca humuli* (DC.) Burr. on several American species of *Rubus* is recorded by Burrill (1, p. 5) and by Salmon (4, p. 49). Burrill (1, p. 6) reports that this parasite does considerable damage

to raspberries. He states further that in many cases only the conidia are produced. It is possible, therefore, that the powdery mildew of raspberry considered in this paper is *Sphaerotheca humuli*. Work is in progress to establish the identity of the mildew, if possible.

SYMPTOMS OF THE DISEASE ON THE LATHAM RED RASPBERRY

The most striking symptom of the disease is the dwarfing of the leaves and the stunting of terminal growth from tip infection (Fig. 1, b and Fig. 2, d). Frequently the tips of canes become infected soon after they emerge from the ground. In such cases the mildew overruns all subsequent growth and the canes seldom attain a height greater than two or three feet. Later infections are relatively less injurious. However, in Minnesota it is not

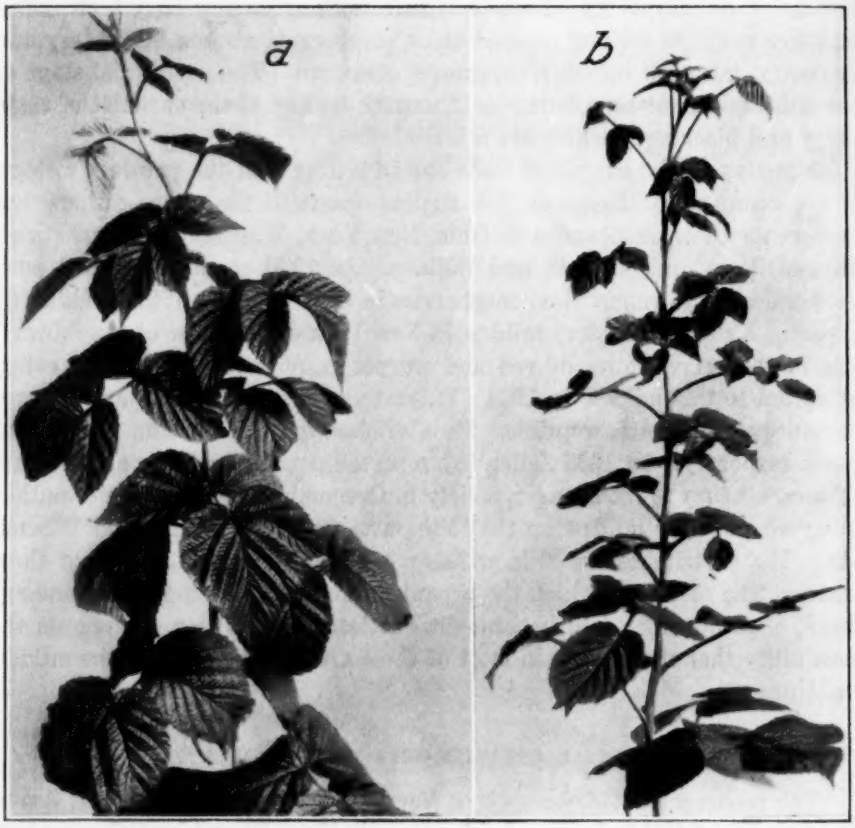

FIG. 1. a. Normal shoot of the Latham red raspberry. b. Latham shoot overrun with mildew: The shortened internodes and dwarfed leaves with upturned margins are characteristic of tip infection.

unusual in late summer to see Latham plantings in which not a single cane has escaped stunting from tip infection.

In addition to tip infection, local infection of the leaves may occur (Fig. 2, a, b, and c). In such cases the fungus is usually confined to the lower surface of the leaf, although, under favorable environmental conditions, both leaf surfaces, the petioles, and the stems may be covered with sporulating hyphae. Even when restricted to the lower surface of the leaves, the mildew is easily discernible from above by a slight yellowing of the infected areas. Infection of young leaves causes marked local hypoplasia, the yellowed areas being flat and smooth while the rest of the leaf often becomes wrinkled and distorted. As the leaves mature, the infected areas frequently die, turn brown, and drop out, so that the leaves become torn and ragged (Fig. 2, a). When local infections are numerous (Fig. 2, b), the leaves assume a mottled appearance similar to that produced by mosaic. Frequently such mildew infection results in Latham plantings becoming so mottled by late summer that roguing for mosaic is impossible. The resemblance to mosaic is further emphasized by a marked stunting due to this general leaf infection.

The fungus apparently does not form its perfect stage on the raspberry. This may be due to one of several factors, a discussion of which is beyond the scope of this paper. The writers wish, however, to call attention to the fact that under field conditions in Minnesota the mildew is invariably parasitized by *Cicinnobolus cesatii* D By. This fungous parasite changes the white, conidial mass to a grayish-brown color, producing much the same appearance as would result from the accumulation of dust (Fig. 2, c). The conidia disappear entirely. The infected area is then found to be practically devoid of leaf hairs. This area appears dark green in contrast to the whitish, hairy under-surface of the normal leaf (Fig. 2, a). Griffiths (2) found the same parasite attacking *Erysiphe cichoracearum* growing on *Grindelia squarrosa*, and concluded that the *Erysiphe* was unable to produce mature perithecia because of the depredations of the parasite. Whether it is responsible wholly or in part for the failure of the powdery mildew to form perithecia on raspberry, we do not know, although it seems certain that the parasite causes little apparent check of the early spread and development of the mildew.

METHOD OF OVERWINTERING

Studies made by the writers during the past two years have shown that the powdery mildew of raspberry overwinters within the dormant buds in the stunted tips of the canes. The apparent lack of perithecia and short period of conidial viability—from 5 to 21 days in our tests—indicate that the common and perhaps the only method the fungus has

of surviving the winter in Minnesota is by means of mycelium within the buds.

In the fall of 1926 mildew-stunted plants of the Latham variety were dug and placed in trenches out-of-doors. On January 29 twenty-one of these

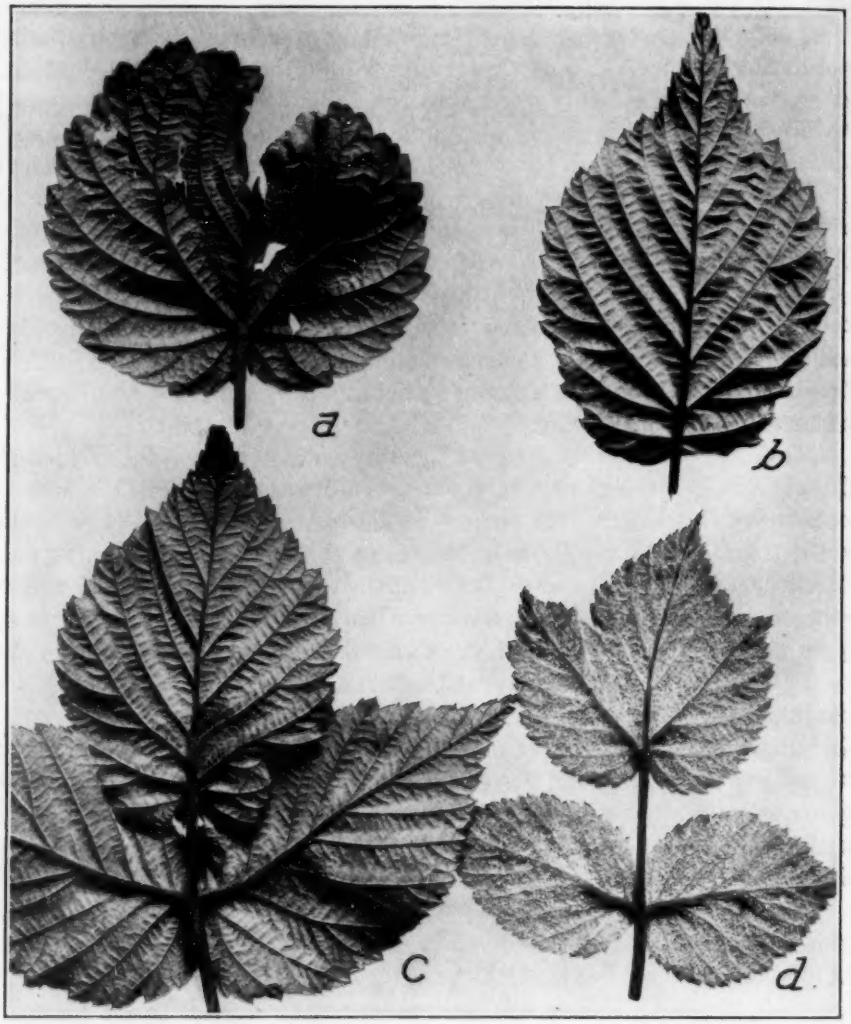

FIG. 2. Characteristic mildew infections on raspberry leaves. a. Old mildew lesion. Infected area devoid of leaf hairs. An area at the tip, killed by mildew, has fallen out. b. Numerous young local infections. Such infections produce leaf mottling similar to mosaic. c. Mildew, near base of leaflets, parasitized by *Cicinnobolus cesatii*. D. By. d. Stunted leaf from tip-infected area of the shoot.

plants were potted and placed in the greenhouse. Mildew developed on 11 of the 21 plants soon after the buds had opened. It developed only on shoots from buds in the stunted tip area of the canes. Infected shoots were entirely overrun by the mildew, which kept pace with the growing point and caused marked hypoplasia of the entire shoot. Such shoots usually died before reaching a length of four inches. They never bore fruit. Unaffected shoots under similar conditions averaged about one foot in length and produced several undersized berries.

On March 4, twenty-five additional plants were taken from the trench, potted, and placed in another greenhouse in which raspberries had not been grown previously. Mildew developed on shoots from buds near the tips of 6 of the 25 plants.

Experiments also were made to determine the percentage of mildew-infected canes in each of the several grades of commercial Latham red raspberry stock. Plants of three grades, Nos. 1, 2, and 3, were obtained from a local nursery. These plants had been cut back, according to the usual nursery practice, to a length of approximately 18 inches. The so-called No. 3 grade, which is not a commercial grade, consisted of plants too small to classify as No. 2 stock. Many mildew runts should be in this class. Judging from the evidence secured in our experiments that only those buds near the tips of the canes were infected, one would expect little or no mildew in the No. 1 stock because two or three feet of the canes had been cut off. Some mildew would be expected in the lighter No. 2 stock which had been cut back less severely. More canes with infected buds would be expected in the No. 3 stock. Mildew developed on none of the 50 No. 1 plants that were potted and placed in the greenhouse. On the other hand, 5 out of 50 No. 2 plants and 7 out of 50 No. 3 plants developed mildew when grown under similar conditions.

As a further check on the method of overwintering, the development of the disease was observed in the field in the spring of 1927. The mildew first appeared on shoots from near the tips of the larger canes and on shoots from buds at any position on the small, badly stunted plants. The first infections of this type were observed May 14, on shoots less than two inches in length. At this time the shoots were entirely overrun by the mildew. Secondary leaf infections were not observed until June 6, more than three weeks later.

It is clear from field studies that infected buds are located chiefly on areas of the canes which have been overrun with mildew the previous season. A few cases were found in which mildew developed from buds on the basal portion of large canes. Such infections can not be accounted for on the basis of tip infection. In these cases the buds were always located near the

base of very large canes which either had sent out lateral branches or showed a tendency to do so. It would seem that such buds might be stimulated to partial growth during the late summer, become infected, and then, due to a change in environmental conditions, again become dormant. Studies made during the summer of 1927 support this hypothesis. Several such buds were found covered with a sporulating growth of mildew. These buds stood well out from the cane, as contrasted to the snugly adhering dormant buds; and the bud scales were partly open, although no terminal growth was evident. Observations made later showed that these buds apparently resumed dormancy.

While the evidence from the above experiments is entirely observational, it leaves little doubt of the fact that the mycelium overwinters in the buds. Based upon similar evidence, mycelial overwintering has long been suggested in the case of a number of powdery mildews, including *Sphaerotheca pannosa*, *Uncinula necator*, *Podosphaera oxyacanthe*, and *P. leucotricha*. Recent work by Woodward (9) on the mode of perennation of *P. leucotricha* has proved definitely that this mildew overwinters by means of hyphae and haustoria within dormant apple buds. The same condition probably exists in the powdery mildew of raspberry. Histological studies are now in progress to ascertain more definitely whether this is true. It would appear from the evidence available that bud perennation may be a common method of overwintering in those powdery mildews that overrun terminal growth. The practical significance of this method of overwintering lies in the tremendous advantage gained by the pathogene through the early production of relatively enormous amounts of inoculum at a time when the host is particularly susceptible, that is, when new growth is being most abundantly formed.

SPRAYING AND DUSTING EXPERIMENTS

The serious epidemic of powdery mildew in Latham plantings throughout Minnesota in 1925 resulted in numerous requests for information regarding control. Spraying and dusting experiments were planned for the 1926 season, therefore, to secure data on the effectiveness of various fungicides in controlling the mildew. The manner in which the fungus overwintered was not known at this time, so no attempt was made to reduce the amount of inoculum by cultural methods.

The spraying and dusting experiments were made in a 25-acre propagative planting of the Latham variety which was known to have been heavily mildewed in 1925. All of the sprayed and dusted plots received a delayed dormant wash of 1–10 lime sulfur. This was followed in one plot by nine applications of 4–4–50 bordeaux mixture, in another by nine applications

of 1–40 lime sulfur, in a third by the same number of applications of copper-lime dust, and in a fourth by nine applications of sulfur dust. Both liquid sprays and dusts were applied at weekly or bi-weekly intervals, depending upon the weather conditions. The sprays were applied by means of a power sprayer which was fitted with three nozzles to the row and delivered the spray at a pressure of 200 pounds to the square inch. The dusts were applied with a power duster.

At the end of the season, the entire field was uniformly infected and stunted. The check plots were as good as, and, in a few cases, better than, the sprayed and dusted plots. Considerable foliage injury occurred in the lime sulfur plots from blossoming time until the end of the season. Prior to the blossoming period no injury was apparent. Sulfur dust caused a similar burning, but to a lesser degree. Hot and dry weather during most of the summer served to accentuate injury by the sulfur compounds. Neither bordeaux mixture nor copper-lime dust caused appreciable foliage injury.

CONTROL BY CULTURAL PRACTICES

Ten applications of fungicide having failed to control mildew in 1926, it was recognized that if spraying or dusting were to be successful, some method of reducing the amount of inoculum was necessary, especially in plantings of the wide-row propagative type. Field observations of the development of the disease during the 1926 season had indicated the probability of bud perennation, and the experiments discussed above were planned to ascertain whether this occurred. If this were the common method of overwintering, then eradication of the pathogene during the dormant season would be a practical and effective control measure. The practical application of this control measure requires adjustment to two sets of growing conditions: (*1*) those of propagative plantings, and (*2*) those of fruit plantings.

Mildew in Propagative Plantings

Mildew is much more destructive in wide-row plantings of the propagative type, owing to several factors: (*1*) the relatively slow growth of the canes because of plant competition, (*2*) the excellent opportunity for all tips to become infected because of the abundance of inoculum made possible by the matted rows, and (*3*) the poor air drainage within such rows. In such plantings few, if any, tips escape infection. Many are infected soon after they emerge from the soil. Such plants will usually grade No. 2 or lower, which means a direct economic loss to the grower. Of equal or greater importance to the grower is the ragged appearance of the planting.

Buyers have frequently turned from mosaic-free but badly mildewed plantings with the remark that they were not on a par with their own condemned stock. Plants in some such plantings average less than 2 feet in height at the end of the season in contrast to shoulder-high, mildew-free plantings of the same variety.

A method of eradicating mildew from plantings of this type was suggested by the practice of "clean-digging" already in use in some Minnesota nurseries. This practice is to dig all of the canes in the row each fall and to permit the new row to come up the following spring from underground parts in the inter-row space. Rows are thus alternated in the planting every other year; the cultivated inter-row space of one year becoming the row of the next year. Experiments were planned for the 1927 season to determine whether the practice of clean-digging could be adapted to mildew control.

The 25-acre field of Latham variety which had been used for the spraying and dusting experiments during the summer of 1926 was dug clean in the fall of that year, although no attempt was made to remove small, immature plants unfit for sale as propagative stock. It was assumed that most of these would winter-kill. On the contrary, however, many of them survived the winter, which was of average severity, and when growth started in the spring, shoots infected with mildew developed from these canes at close intervals over the entire field. In spite of this fact, the mildew developed more slowly in this planting than it had the previous season. This may have been due in part to weather conditions, but the eradication of a large number of infected buds in the digging of all mature canes was undoubtedly an important factor. However, as a demonstration of control this experiment was considered to be a failure.

In another part of the state, a second field was dug clean in the fall of 1926. All the canes suitable for sale as propagative stock were dug, and, in addition, a strip through the center of the field was harrowed until all of the young growth had been covered up or destroyed. In 1927, plants in the area which had been harrowed remained free from mildew, other than a few local infections, until late summer. The strips on both sides were abundantly infected early in the season. The plants in the harrowed area were also much freer from cane diseases, such as grey-bark (*Mycosphaerella rubina*) and anthracnose (*Plectodiscella veneta*).

This experiment has demonstrated that powdery mildew can be controlled in wide-row propagative plantings, by the practice of clean-digging even though there are other infected plantings nearby. In such cases, spread from outside sources of inoculum is apparently so slow that very little stunting from mildew results.

Mildew in Fruit Plantings

Observations made during the past three seasons in plantings in which the Latham variety was being grown for fruit have shown that powdery mildew is not a serious problem in plantings in which either the narrow hedge or restricted hill system is used. In localities where severe stunting has occurred in wide-row propagative plantings, little or no stunting has been noted in fruit plantings in which the hedges have been narrowed down to a single row of plants or the hills restricted to from three to five canes. The canes in such plantings have maintained a growth level well above the height at which they are allowed to fruit. This permits sufficient pruning-back during the dormant season each year to insure removal of most, if not all, of the infected tips. In addition, it is necessary to remove and destroy all stunted plants and the late season growth which, as was shown in the authors' clean-digging experiments in propagative plantings, may live through the winter and serve as centers of infection the following spring.

SUMMARY

1. Destructive epidemics of powdery mildew have occurred in Latham red raspberry plantings throughout Minnesota during the past three seasons. The disease has been reported also from Ohio, New York, Washington, Connecticut, Illinois, Maryland, Oregon, Indiana, Michigan, and Wisconsin.

2. The perithecia of this powdery mildew have not been found in Minnesota, nor by recent observers in any of these other states so far as the writers have been able to determine. In the absence of the perfect stage, it has been impossible to identify the mildew definitely. However, Burrill and Salmon both record the occurrence of *Sphaerotheca humuli* on several American species of *Rubus*, and it is possible that this is the powdery mildew of raspberry discussed in the present paper.

3. Under field conditions in Minnesota, the mildew is invariably parasitized by *Cicinnobolus cesatii* D By. This may account for the failure to form perithecia.

4. The most striking symptom of the disease is the dwarfing of the leaves and the stunting of terminal growth from tip infection. Local infection of the leaves also occurs. In such cases the fungus is usually confined to the lower surface of the leaf but, under favorable environmental conditions, may spread over both leaf surfaces, the leaf petiole, and the stem. When local infections are numerous, the leaves assume a mottled appearance similar to that produced by mosaic.

5. Experiments have shown that the organism can persist throughout the winter in infected raspberry buds which, as a rule, are located in

the stunted-tip area of diseased canes. The apparent lack of perithecia and the fact that spore viability studies have shown that the conidia are very short-lived indicate that perennation within the buds is the common, and perhaps only, method the organism has of surviving the winter in Minnesota.

6. The disease was not satisfactorily controlled either by spraying or by dusting in large-scale field experiments made in 1926.

7. The practice of "clean-digging" has proved to be an effective method of eradicating the pathogene from propagative plantings of the Latham red raspberry in Minnesota. Growers following this practice eliminate almost entirely the stunting effect which results from early tip infections of powdery mildew.

8. Observations during three seasons have shown that powdery mildew is not a serious problem in fruit plantings in which either the narrow hedge or restricted hill system of culture is practiced.

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LITERATURE CITED

1. ELLIS, J. B., and B. M. EVERHART. The North American Pyrenomycetes. 793 pages. Ellis and Everhart, 1892.
2. GRIFFITHS, D. The common parasite of the powdery mildew. Bul. Torr. Bot. Club **26**: 184–188. 1899.
3. LEACH, J. G., and J. L. SEAL. Powdery mildew of raspberries (Abst.). Phytopath. **14**: 61. 1924.
4. SALMON, E. S. A monograph of the Erysiphaceae. Memoirs Torr. Bot. Club. 9. 292 pages. 1900.
5. U. S. DEPT. AGR. Plant Disease Bul., Sup. 14: 101. 1921.
6. ————————. Plant Disease Bul., Sup. 28: 371. 1923.
7. ————————. Plant Disease Reporter, Sup. 39: 80. 1925.
8. ————————. Plant Disease Reporter, Sup. 47: 275. 1926.
9. WOODWARD, R. C. Studies on *Podosphaera leucotrica*. I. The mode of perennation. Trans. Brit. Mycol. Soc. **12**: 173–204. 1927.

PRELIMINARY NOTE ON SNOWBERRY ANTHRACNOSE[1]

M. F. Barrus and James G. Horsfall

The common snowberry, *Symphoricarpos albus* (L.) Blake, var. *laevigatus* (Fernald) Blake, in the vicinity of Ithaca, New York, is severely affected by an anthracnose. Numerous observations have revealed that severe defoliation and a destruction of the berries often accompany the disease. The name "anthracnose" is applied because of the resemblance of the causal fungus to that causing the familiar raspberry anthracnose.

The disease has been observed to be destructive in Delaware, Tioga, and Tompkins Counties in New York State, and the writers have received severely diseased specimens from Arkansas,[2] Iowa, and Wisconsin. Since apparently only a single note[3] regarding the disease has been recorded in the literature, the publication of this paper appears warranted.

Snowberries are widely planted for the beauty of the snow-white fruits which persist after the leaves have fallen until well into the winter, but when plants are affected with anthracnose this character is tremendously impaired. Such losses are, of course, difficult to estimate, but they must be appreciable.

The disease appears on the leaves early in the spring as minute, dark-purple to black spots which enlarge slowly (Plate XVI). The center later becomes light- to dirty-gray in color with a margin of dark-purple to black. Spots are usually circular and sharply delimited from healthy leaf tissue, but their coalescence will often produce a large, ugly, irregular spot which is subject to cracking in the center. When marginal infections kill the tissues early, they cause marked hypoplastic deformations. Frequently the leaves are badly misshapen as a result of such infections. Inconspicuous lesions essentially like those on young leaves appear on flower-buds and leaf-buds before they unfold. Lesions were observed once on the petals of an open flower.

[1] The authors wish to express their indebtedness to Professor H. H. Whetzel, Department of Plant Pathology, Cornell University; to Dr. Anna E. Jenkins, Office of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture, for helpful assistance and criticism during the course of the investigation and for aid in reading the manuscript; and to Mr. W. R. Fisher for making the photographs.

[2] The specimen from Arkansas was secured through the kindness of Mr. William Horsfall, Fayetteville, Ark. Thanks are also due the Office of Mycology and Disease Survey, United States Department of Agriculture, for the contribution of the specimens from Iowa and Wisconsin.

[3] Stewart, F. C. Anthracnose, *Gloeosporium* (?) *sp.* Notes on New York plant diseases, I. N. Y. (Geneva) Agr. Exp. Sta. Bul. 328: 393–394. 1910.

Small, dark-purple to black lesions can be found on the calyx end of fruits shortly after the petals fall (Plate XVII). These spots, which are fimbriately margined, do not appear to go much deeper than the cuticle and the first few layers of cells below. Berries, like leaves, infected when small, become lopsided. The spots appear as circular, somewhat sunken, pink lesions of various sizes on the more mature berries. Frequently an *Alternaria* invades the diseased tissue and causes a watersoaked lesion to develop which speedily involves the whole fruit. Such fruits soon become shriveled, dry, brown mummies. Occasionally late in the season, fruiting branches may be seen that are devoid of any berries except a few which emerged before the inoculum became copious.

In the early spring, the tender shoots often are attacked by the malady. Here the disease is expressed as small, oblong, and somewhat sunken spots having a dark-gray color. The lesions on the shoots are rarely, if ever, more than two millimeters in length.

Considerable difficulty in isolating the causal organism was experienced at first, before its identity had been established. In the early part of the investigation only an *Alternaria* and a *Gloeosporium* were isolated from diseased areas. Puncture inoculations with the *Alternaria* produced the watersoaked lesions described previously, but these were in no way comparable with anthracnose lesions. Atomizing the Gloeosporium conidia on to berries did not produce the disease. During the summer of 1926 an organism was isolated which was later identified as a species of *Sphaceloma* by Miss Anna E. Jenkins, who based her determination on gross cultural features. This fungus was obtained by spore dilution and from plantings of diseased tissue in tubes of agar. Two additional isolations of it were obtained in 1926, and during the season of 1927 it was obtained eight times from diseased leaves, twigs, and berries.

The causal organism is peculiar, for on standard culture media such as potato-dextrose agar it has almost none of the hyphal growth usually associated with fungi in culture. It grows very slowly, forming a tough mat of mycelium which appears characteristically folded and ridged on the surface with but little submerged growth. Superficially the growth looks like gelatine. An individual thallus will attain a size of one centimeter or so in diameter and then cease to grow, so that it has not been known to cover completely an agar slant. On potato or oat agar, the mycelium is predominantly red. In old cultures, the color may become dusky-slate-violet,[4] but in more recent transfers the color is frequently light enough to be called vinaceous-buff. The color will usually diffuse from the mycelium

[4] The color readings of cultures are based on RIDGWAY, R. Color standards and color nomenclature. Washington, D. C. 1912.

and impart a beautiful dark-red shade to the medium of cultures a month or so old.

Because spores were not available in pure culture at first, mycelium covered with moist cotton on young leaves in the greenhouse served as inoculum. In 10 days the minute infections were found. The fungus was reisolated 44 days after inoculation. Using the same technique, infection on young berries was observed in 6 days. The pathogene was successfully reisolated 41 days later. A third successful inoculation of snowberry leaves was accomplished later using spores subsequently produced in culture from the fungus reisolated from the first mycelial inoculation. In this case infection was apparent in 4 days. Plate XVI shows these leaves 39 days after the spores were sprayed on them. On this date the organism was reisolated and presented cultural characters indistinguishable from those originally observed.

Very little work upon control has been accomplished. An experiment in dusting bushes five times during the season of 1927 with copper lime dust, although inconclusive, gave some evidences of control. More attention to timing the applications should produce better results.

Cultures of *Sphaceloma fawcettii* Jenkins, *Sphaceloma ampelinum* DeBary, and *Plectodiscella veneta* Burkholder were secured from Miss A. E. Jenkins[5] and compared simultaneously with the snowberry anthracnose fungus. These were found very similar, but nevertheless certain constant cultural characters, such as type of growth and color, indicated that the last is different from any of the others. Furthermore, none of these fungi infected young snowberry fruits, when at the same time this fungus did induce infection.

Because of the resemblance of the snowberry anthracnose fungus in culture to *Sphaceloma ampelinum* DeBary,[6] the type species of the genus, it has been considered best to place it in the form genus *Sphaceloma*. Since no technical name appears to have been applied to the fungus, it is here described as a new species.

Sphaceloma symphoricarpi n. sp. Acervuli sub-cuticular to sub-epidermal becoming erumpent, solitary, gregarious, or confluent, irregular, from 50 to 300 μ in diameter, on leaves, fruits and stems, on leaves epiphyllous; stroma pseudo-parenchymatous above, prosenchymatous below; conidiophores at first hyaline then dark, unbranched, characteristically sharp-pointed, closely compacted, arising perpendicularly, often in tufts, from surface of stroma; conidia terminal, embedded in a gelatinous matrix, oblong-ellipsoid, 4.0×8.0 μ (3.0–5.4 × 6.0–9.0 μ) (observed in culture),

[5] JENKINS, A. E. The citrus-scab fungus. Phytopath. **15**: 99–104. 1925.

[6] DEBARY, A. Ueber den sogenannten Brenner (Pech) der Reben. Annal. der Oenol. **4**: 165–167. 1873.

typically bi-guttulate, one oil drop in each end, continuous, hyaline, later becoming slightly dark.

Habitat: Parasitic on *Symphoricarpos albus* (L.) Blake, var. *laevigatus* (Fernald) Blake; spots on leaves dirty-gray in center with dark-purple margin; on fruits at first dark-purplish to black with fimbriate margin, later formed spots becoming pink and sunken; on succulent stems small, oval, depressed lesions gray in center with dark margin.

Type locality: Forest Home, Ithaca, New York.

Distribution: New York, Wisconsin, Iowa, and Arkansas.

Material examined: Ithaca, N. Y., M. F. Barrus and J. G. Horsfall, C. U. Pl. Path. *15252* (type); Ithaca, N. Y., 1927, M. F. Barrus, C. U. Pl. Path. *15768*; Ithaca, N. Y., 1927, J. G. Horsfall, C. U. Pl. Path. *16250*, (artificial inoculation in greenhouse); Walton, N. Y., 1927, A. E. Jenkins, C. U. Pl. Path. *16305*; Fayetteville, Ark., 1927, W. R. Horsfall, C. U. Pl. Path. *16306*; Madison, Wis., 1927, R. E. Vaughan, C. U. Pl. Path. *16307*; Ames, Ia., 1927, R. J. Haskell, C. U. Pl. Path. *16308*.

These specimens, as indicated by their accession numbers (C. U. Pl. Path. No.), are to be found in the herbarium of the Plant Pathology Department of Cornell University, Ithaca, N. Y.

Authentic specimens have been deposited in the following herbaria: Pathological Collections, Bureau of Plant Industry, Washington, D. C.; New York Botanical Garden, New York, N. Y.; Royal Botanical Garden, Kew, Surrey, England.

SUMMARY

1. Snowberries are seriously affected with anthracnose in the vicinity of Ithaca, N. Y. The disease has been reported also from Wisconsin, Iowa, and Arkansas.

2. The symptoms of the disease are described, and Koch's rules of proof have been fulfilled for the causal organism.

3. The pathogene is described as a new species, *Sphaceloma symphoricarpi*.

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EXPLANATION OF PLATES

PLATE XVI

A. Upper surface of young leaf of *Symphoricarpos albus* var. *laevigatus* showing lesions caused by *Sphaceloma symphoricarpi*.

B. Lower surface of young leaf showing lopsidedness caused by infection.

C. Lower surface of old diseased leaf.

D. Same as C, another leaf.

E. Upper surface of old diseased leaf. Note cracking above.

F. Small sunken lesions on stem.

G. Recent infections on succulent twig.

H. Inflorescence showing minute lesions on unopened flower buds.

I. Leaves 39 days after being inoculated with spores. Note the dead apical leaf and its deformed mate.

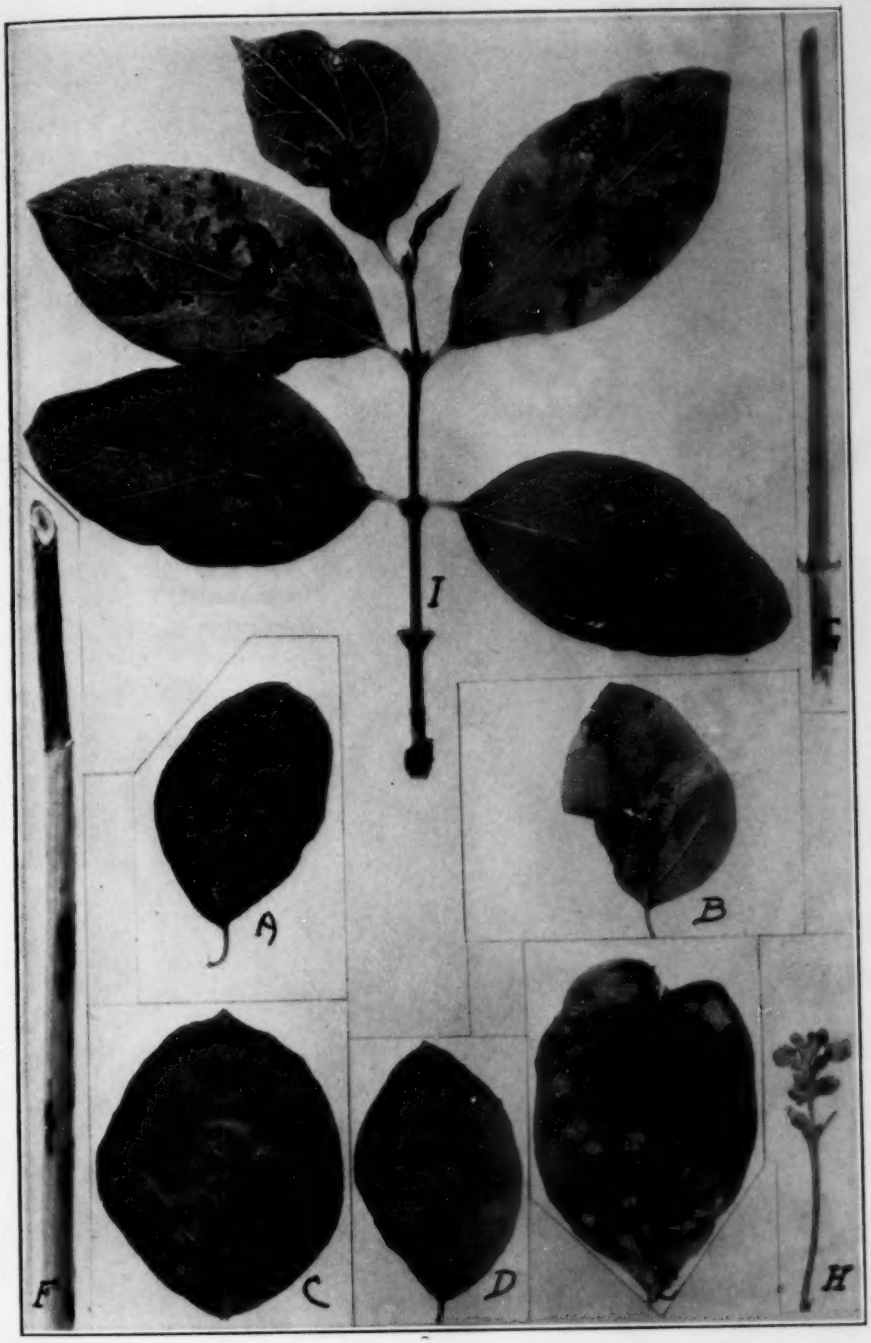
PLATE XVII

J. Fruit cluster showing late-formed lesions.

K. Fruit cluster showing early fimbriate-margined infections. Note deformed berries.

L, M, N. Berries late in season mummifying because of *Alternaria* infection following *Sphaceloma*.

O, P, Q, R. Individual berries showing later infections. At Q, note minute black acervuli in the lesion.



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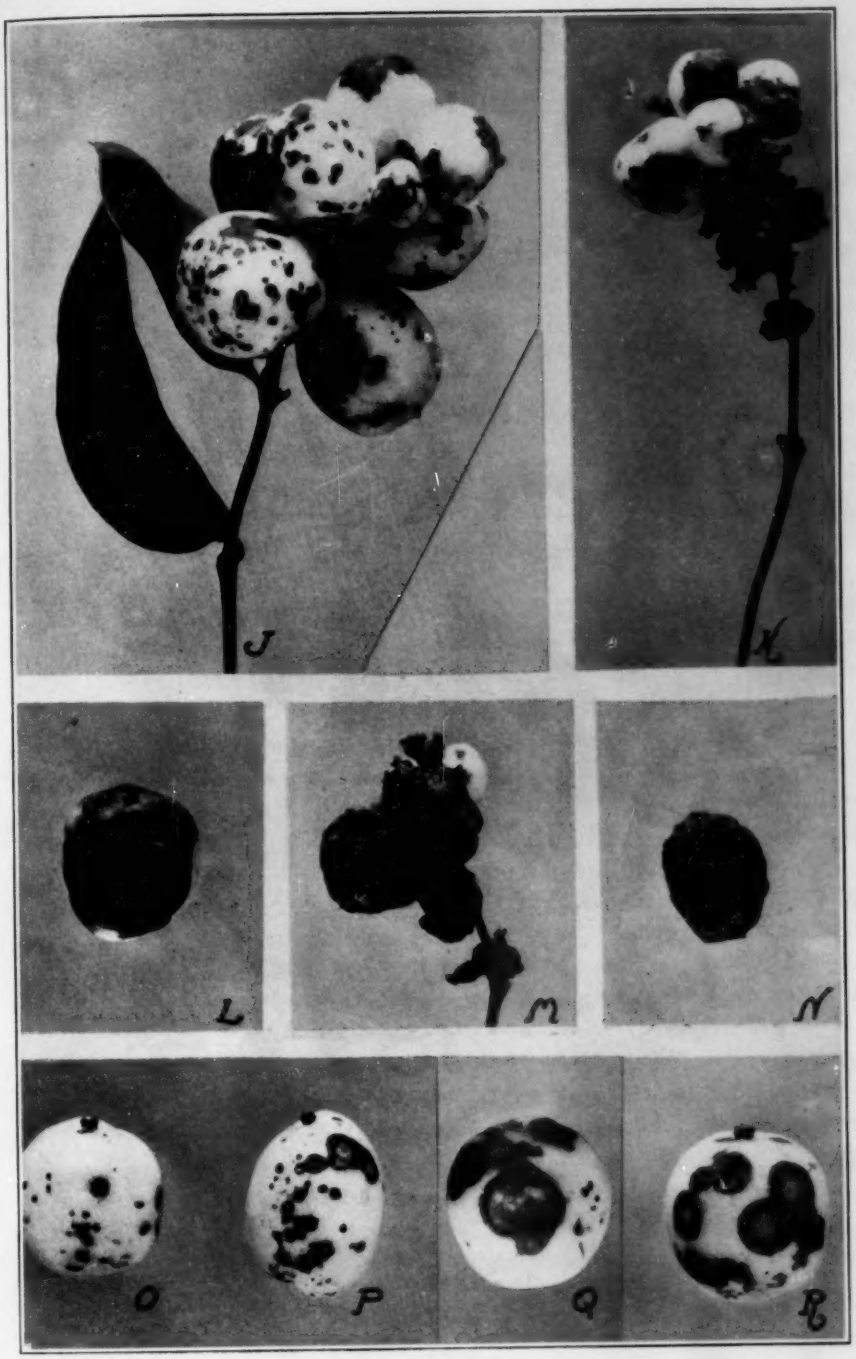

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THE DEMONSTRATION OF BACTERIA IN PLANT TISSUES BY MEANS OF THE GIEMSA STAIN[1]

WILLIAM H. WRIGHT AND VLADIMIR SKORIC

The staining of bacteria in animal tissues has received so much more attention than those in plants that correspondingly better methods have been developed. In this respect the animal pathologist is more fortunate than the phytopathologist. It seems quite reasonable to suppose that some of the animal methods might be adapted for use in similar studies with plants.

A review of the literature shows that fuchsin has been the most generally used of the stains for bacteria in plant tissues. Dale (2) obtained good results using Diamond Fuchsin with light green as a counterstain. While making a study of the symbiotic bacteria in the leaves of the *Rubiaceae*, Faber (4) obtained variable success but found fuchsin as satisfactory as any stain tried.

The differential nature of the Gram stain offers much more of value for the study of bacteria in animal tissues than it does with plant tissues. Smith (14) recommends the Gram stain for the staining of bacteria in plant tissues when the bacteria are Gram positive. However, comparatively few bacteria pathogenic for plants are Gram positive.

Other stains, such as iron haematoxylin and Flemming's triple stain, have often given good results. Satisfactory preparations are sometimes obtained with water soluble nigrosin. Methyl violet has been found very satisfactory for the staining of *Bacillus tracheiphilus* in cucumber tissue. Preliminary treatment in tannin water is supposed to prevent excessive staining of the host tissue. Riker (10) used dilute carbol fuchsin to stain the bacteria of crowngall in plant tissue. Light green was used as a counter-stain. Magrou (8) successfully uses pyronine and methyl green on this material.

The variable results obtained when attempts are made to stain bacteria in plant tissue emphasize the need for more study of the staining processes for such material. The combining power of acid and basic dyes with pure proteins has been shown by Loeb (6). More recently Robbins (11) has made use of the same principle in the determination of the isoelectric range of plant tissue. Following this general procedure Naylor (9) developed a method of differential staining for sections or foot tips of various plants.

[1] Published with the approval of the Director of the Agricultural Experiment Station, University of Wisconsin.

By staining with acid and basic dyes of contrasting colors and washing with buffer mixtures at definite hydrogen ion concentrations, differences in the power of different parts of the cells to retain the dyes resulted in differential staining. The results of these investigators, as shown by the absorption of dyes, indicate that the isoelectric range for most plant tissues is between pH 4.6 and pH 5.0. These values are apparently much higher than those found for some of the bacteria causing plant diseases. In a recent study of the bacteria causing wilt of bean (*Bact. flaccumfaciens*), blight of bean (*Bact. phaseoli*), and pustule of soybean (*Bact. phaseoli sojense*), Sharp (13) found the isoelectric range of each of these organisms to be within pH 1.2 and pH 3.0.

If combination of the protein constituents of cells with dyes is as dependent upon the hydrogen ion concentration of the dye-protein mixtures as recent experiments seem to show, it should be possible, by selecting acid and basic dyes of contrasting colors, to differentiate bacteria in tissues from the tissue constituents.

THE GIEMSA STAIN

Giemsa's stain is a mixture or compound dye. Zoologists and animal pathologists have often used mechanical mixtures of dyes for differential staining. Aside from the contrasting colors due to the selective powers of the dyes in the mixtures, there may result, from the mixing new dyes compounds with very valuable staining properties.

Romanovski (12) was one of the first to suggest the use of a mixture of acid and basic dyes for staining protozoa. The stain was later modified by Giemsa (5) and recommended for cytological and blood work. This stain was used by Dobell (3) in an extensive study of the cytology of bacteria.

The Giemsa stain is usually prepared from Azure I (Giemsa) containing a variable mixture of Azure A (asymmetric dimethyl-thionin) and Azure B (tri-methyl thionin). Azure II (Giemsa) is a mixture of Azure I (Giemsa) with methylene blue. These two dye mixtures are prepared for use in very pure methyl alcohol with eosin (tetrabrom fluoresceein). Recently MacNeal (7) has developed a simple method of preparing methylene Azure A (asymetric di-methyl-thionin) which may be used instead of the less stable methylene azure.

THE STAINING OF BACTERIA IN PLANT TISSUES WITH THE GIEMSA STAIN

Several staining methods were tried in the study of pathogenic bacteria in plant tissues and soybean nodule bacteria in roof nodules of soybean. When compared with other methods, the Giemsa stain was found to give the best results. The purest grade of neutral methyl alcohol was used and very pure water added when dilution was necessary. A ready prepared,

dry form of the commercial dye was used to prepare a fresh solution each time. Several batches of the dye solution, when tested potentiometrically, were found to have pH values varying from 4.2 to 4.5. Sections gave the best staining results when treated with several changes of buffer solutions, near pH 6.0 for an hour or more. Reactions more alkaline tended to overstaining with the Giemsa stain. As a counterstain 2 per cent safranin in absolute alcohol was used.

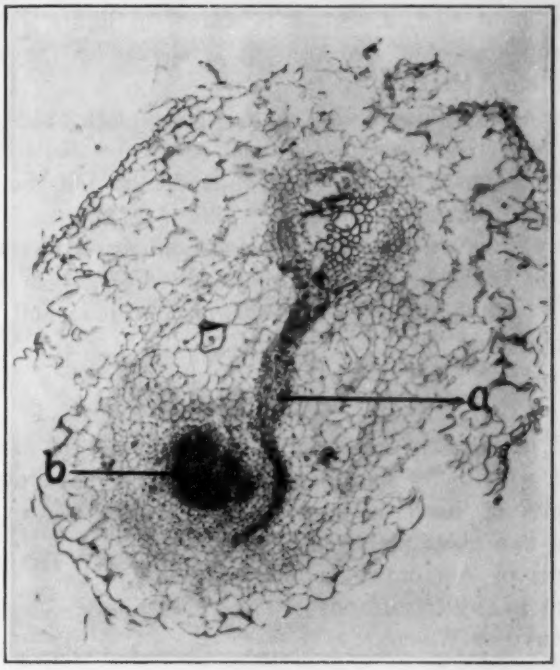

FIG. 1. Section of a soybean root nodule. The extension of the vascular structures from the root to the nodule may be seen at *a*. The cells showing dark at *b* are filled with deeply stained bacteria. Magnification 100×.

Tissues fixed by several methods were found to give very satisfactory results. The procedure of Bensley (1) as used for chondriosomes was used with good results.

Material embedded in paraffin was cut serially into sections of 4–6 micra in thickness. These, after the usual fixation and stretching, were treated as follows:

1. Remove paraffin in xylol.
2. Absolute, 80, 50, 30, and 15 per cent alcohol.
3. Wash in water and treat for an hour in several changes of buffer solutions near pH 6.0.

4. Stain in Giemsa stain for 10 to 15 minutes.
5. Wash in absolute alcohol until the tissues are nearly free from stain but the bacteria retain it. This varies with the pH of the material.
6. Dip the slides 3 to 6 times in the safranin solution.
7. Wash in absolute alcohol.
8. Xylol and then balsam.

The bacteria are deep blue, and, as in the case of the soybean nodule bacteria, the areas occupied by them can be seen with the naked eye. The photomicrograph of such a section is shown in figure 1. The general microscopic appearance of two sections is shown by the camera lucida drawings in Plate XVIII.

The bacteria are stained deep blue, plasma light blue, nuclei pink, nucleoli blue, and cell walls red. In cases where the tissue had undergone change, due to bacterial action, it was stained blue, but the bacteria were a deeper blue and sharply defined.

By means of this method, *Pseudomonas radicicola* was stained in the root nodules of soybean, *Mycobacterium rubiacearum* in the leaves of *Psychotria bacteriophylla*, *Pseudomonas pisi* in pea, and *Pseudomonas phaseoli* in bean tissue.

SUMMARY

A method of staining bacteria in plant tissues is described, using Giemsa's stain with buffer solutions and safranin as a counterstain.

Four species of bacteria were stained, including two intracellular symbionts and two plant pathogens.

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LITERATURE CITED

1. BENSLEY, R. R. Studies on the pancreas of the guinea pig. Amer. Jour. Anat. **12**: 297–388. 1912.
2. DALE, E. A bacterial disease of potato leaves. Ann. Bot. **26**: 133–154. 1912.
3. DOBELL, C. C. Contributions to the cytology of the bacteria. Quart. Jour. Mic. Sci. **56**: 395. 1911.
4. FABER, F. C. Das erbliche Zusamenleben von Bacterien und tropischen Pflanzen. Jahrb. wiss. Bot. **51**: 285–375. 1912; and **54**: 243–264. 1914.
5. GIEMSA, G. Eine Vereinfachung und Vervollkommnung meiner Methylene-azur-Methylene blau-eosin Färbemethode zur Erzielung die Romanowsky-Nochtsen Chromatinfarbung. Centralbl. Bakt. Abt. I, **37**: 303–311. 1904.
6. LOEB, J. Proteins and the theory of colloidal behavior. 292 pp. New York. 1922.
7. MACNEAL, W. J. Methylene violet and methylene azure A and B. Jour. Infect. Dis. **36**: 538–546. 1925.

8. MAGROU, J. Recherches anatomiques et bacteriologiques sur l'cancer des plantes. Ann. Inst. Pasteur. **41**: 785–801. 1927.

9. NAYLOR, E. E. The hydrogen ion concentration and the staining of sections of plant tissue. Amer. Jour. Bot. **13**: 265–275. 1926.

10. RIKER, A. J. Some relations of the crowngall organism to its host tissue. Jour. Agr. Res. **25**: 119–132. 1923.

11. ROBBINS, J. W. An isoelectric point for plant tissue and its significance. Amer. Jour. Bot. **10**: 412–440. 1923.

12. ROMANOVSKI, D. L. Zur Frage der Parasitologie und Therapie der Malaria. St. Peterab. Med. Wochenschr. **16**: 297–302, 307–315. 1891.

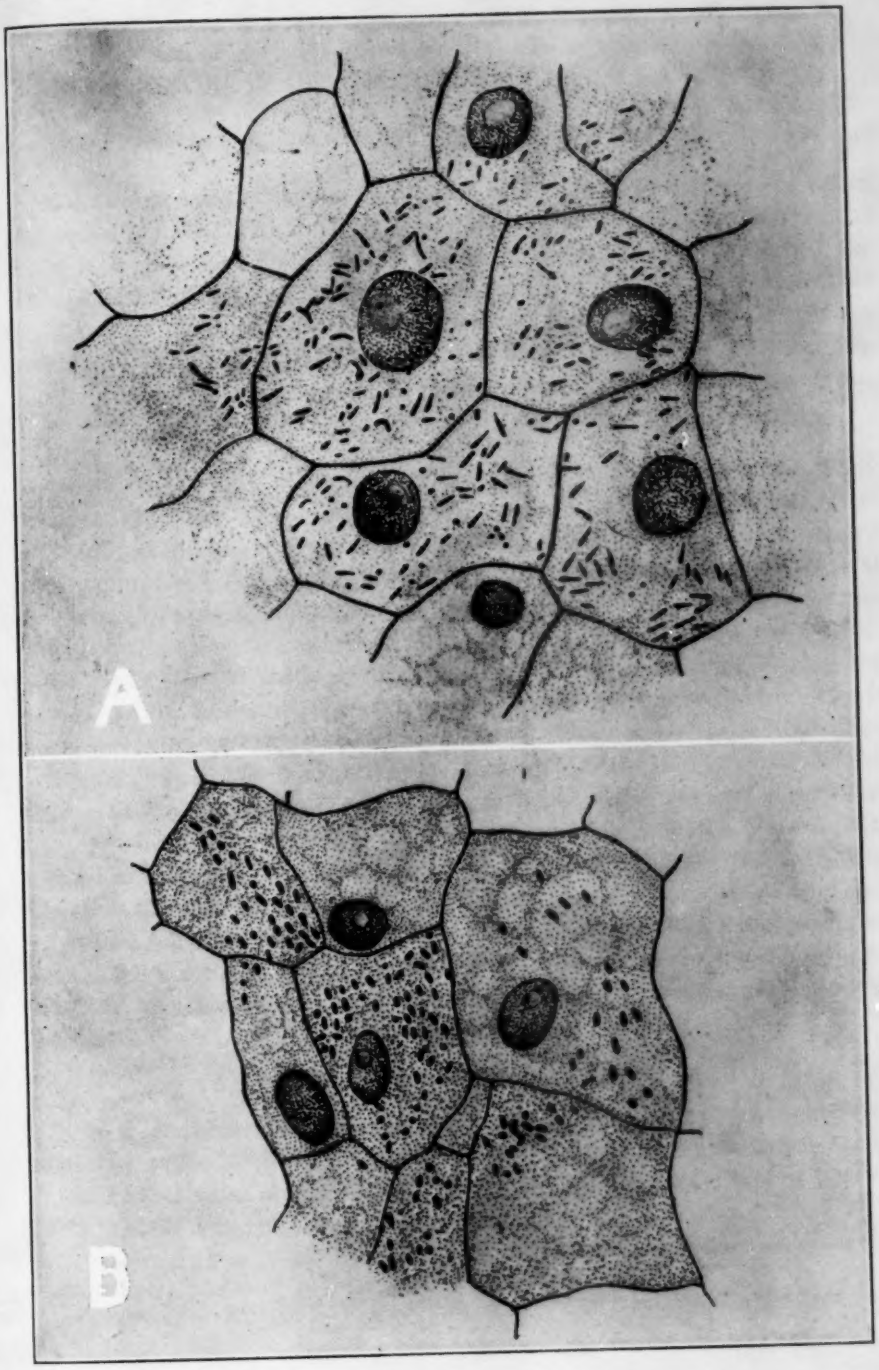
13. SHARP, C. G. Virulence, serological and other physiological studies of *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*. Bot. Gaz. **8**: 113–144. 1927.

14. SMITH, E. F. An introduction to the bacterial diseases of plants. 688 pp. W. B. Saunders Co., Philadelphia. 1920.

EXPLANATION OF PLATE XVIII

A. Typical area in slightly infected part of a nodule of *Soja max*. Section cut 4 micra in thickness. Camera lucida drawing. Magnification 2000×.

B. Typical area in part of a nodule of a leaf of *Psychotria bacteriophylla*. Camera lucida drawing. Magnification 2000×.



UNIV. OF MICH.

STORAGE ROTS OF CRANBERRIES IN THE 1927 CROP[1]

NEIL E. STEVENS AND HENRY F. BAIN

Continuing studies begun in the fall of 1926,[2] the writers have again attempted to measure quantitatively the actual storage rot caused by various fungi in cranberries from different growing regions. The results of the storage tests with the 1927 crop are given herewith. While for reasons that will be pointed out, the results are not strictly comparable in all respects with those of the 1926 tests, they furnish a fairly complete and accurate picture of the actual storage rot in the cranberry crop of the current year.

METHODS

The methods were substantially the same as in the previous test, fully described on page 650.[2] In presenting the results, however, a somewhat different method has been used, and the data for 1926 have been recalculated for comparison. The height of each column, figure 1, represents the total percentage of spoilage to January 1 in that particular lot of berries. Fractional parts of the total spoilage initiated by different causes were determined by means of cultures made at intervals of one month during the storage period, as described in the earlier paper. At the beginning of each two-weeks' storage test the percentage rot in the stock box was determined by actual count of representative samples of berries. Only sound berries were used for the test lot. In the present calculations, values for each test lot were corrected to represent the percentage of spoilage on a basis of total berries in the stock box rather than of sound berries in the test lot. The percentage values for an entire month were then determined by applying the values found in the two-weeks' test to the actual spoilage that occurred during the month, as determined by the difference between two consecutive counts of spoilage in the stock boxes. These monthly values were summed up to give the totals represented in figure 1.

RESULTS

The chief interest of these tests lies, of course, in the differences between 1926 and 1927. The results are summarized in table 1 and figure 1. In figure 1 the results are shown individually for the separate lots of berries, while in table 1 the lots are averaged.

[1] Investigation conducted cooperatively by the Office of Fruit Diseases, Bureau of Plant Industry, and the Wisconsin Department of Agriculture.

[2] STEVENS, N. E., and H. F. BAIN. Storage rots of cranberries in the 1926 crop. Phytopath. 17: 649–655. 1927.

Under the conditions of the tests, the Howes and McFarlin cranberries from Massachusetts, New Jersey, and Oregon showed somewhat more rot in 1927 than in 1926. The difference was greatest in berries from Massachusetts, and least in those from Oregon. Berries from Wisconsin, on the other hand, kept somewhat better in 1927 than in 1926. Examination of the graphs and of the first two columns of table 1 shows that the difference between 1926 and 1927 in Massachusetts and New Jersey is distributed among various causes as follows: There was a slight increase in sterile breakdown and in the loss from end rot; and there was a marked increase in the loss from *Guignardia* and *Glomerella*.

CAUSES OF THE DIFFERENCE IN LOSS IN 1926 AND 1927

After a careful study of the conditions of the experiment, the writers have been forced to the conclusion that the difference in results obtained

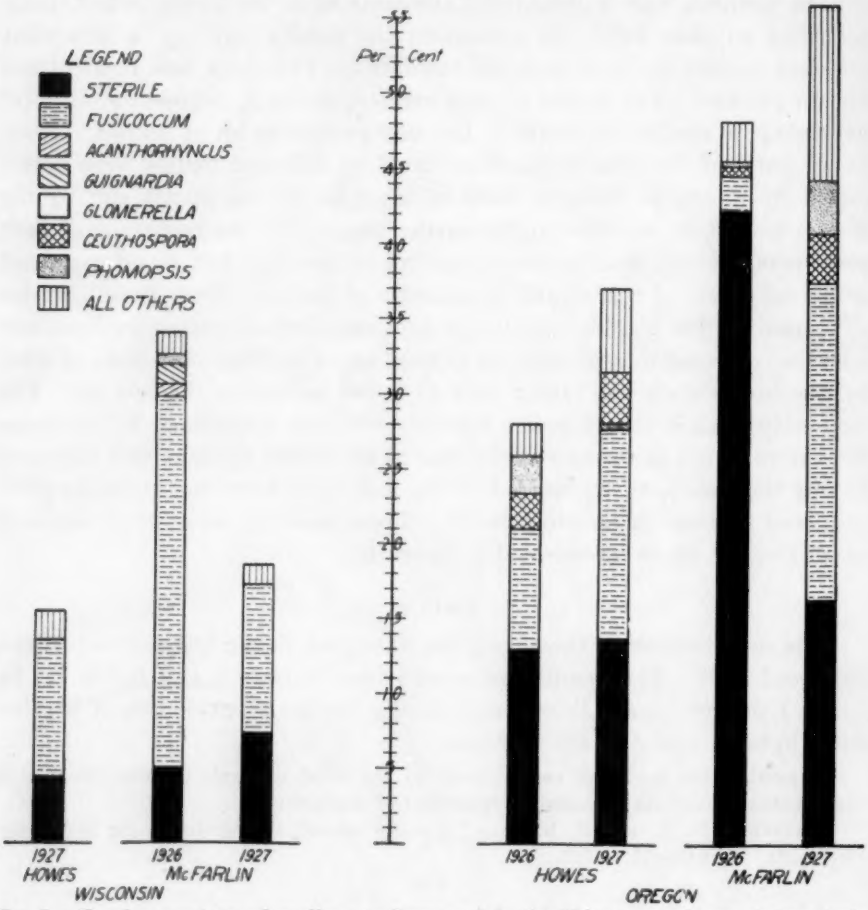


FIG. 1. Total percentage of spoilage to January 1 in the Chicago cranberry storage tests of 1926–7 and 1927–8, with proportions initiated by different fungi and sterile breakdown shown by suitable conventions.

TABLE 1.—*Percentage of loss due to different fungi and other causes in the Howes and McFarlin varieties of cranberries from Massachusetts, New Jersey, Wisconsin and Oregon, in the 1926 and 1927 Chicago storage tests, to January 1 of the following year*

Cause of loss	Average loss in per cent in both varieties			
	Massachusetts and New Jersey		All four states	
	1926	1927	1926	1927
Sterile	7.41	9.39	12.80	9.90
Fusicoccum putrefaciens Shear	9.99	11.35	10.80	12.50
Acanthorhyncus vaccinii Shear	1.75	1.40	1.20	0.80
Guignardia vaccinii Shear	3.11	15.66	2.00	7.80
Glomerella rufomaculans vaccinii Shear	0.77	3.42	0.40	1.70
Ceuthospora lunata Shear	—	—	0.40	0.80
Phomopsis sp.	0.81	0.72	1.00	0.80
Total spoiled	26.96	45.27	31.10	38.60

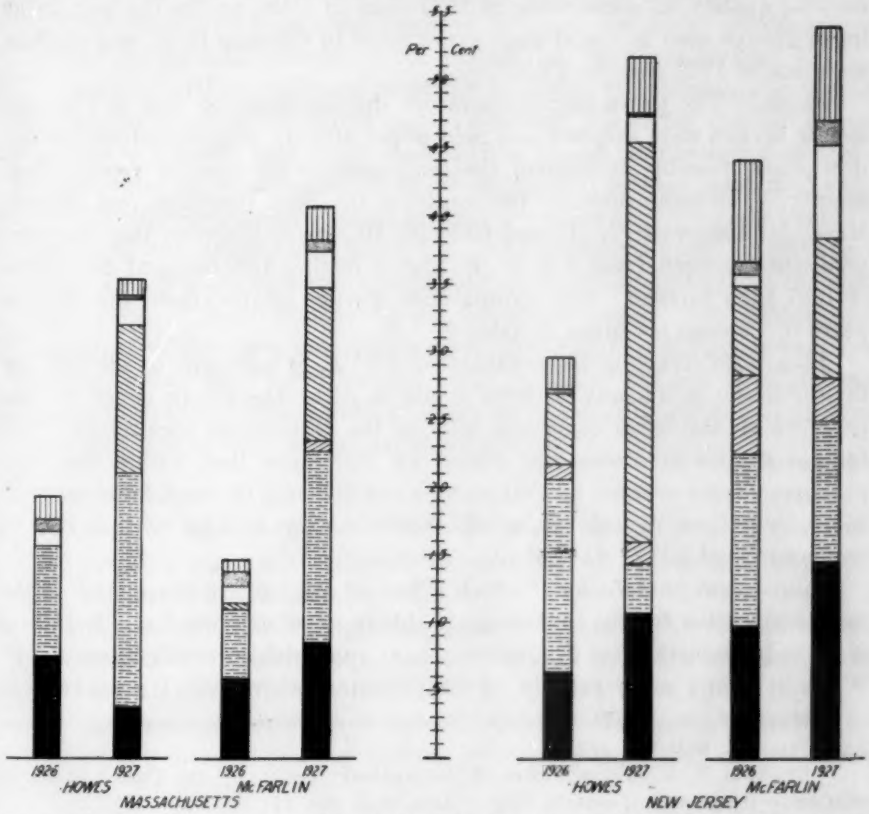


FIG. 1. (continued.) Total percentage of spoilage to January 1 in the Chicago cranberry storage tests of 1926–7 and 1927–8, with proportions initiated by different fungi and sterile breakdown shown by suitable conventions.

from Masschusetts and New Jersey berries in the two years is due to the difference in the temperature at which the berries were shipped and stored. The evidence on this point may be summarized as follows:

First—The condition of the berries as shown by holding tests in 1926 and again in 1927. A number of lots of berries of different varieties from various bogs in Plymouth County, Massachusetts, were assembled and held in basement storage at East Wareham; a somewhat smaller number of lots of New Jersey berries in a field laboratory in Toms River. The Massachusetts lots were sorted and counted on November 15 of both years. The New Jersey lots were sorted and counted November 12. An average of 14 strictly comparable lots from Massachusetts gave 12.3 per cent spoilage in 1926 and 7.9 per cent spoilage in 1927. From these results it is apparent that the condition of Massachusetts berries when harvested must have been slightly better in 1927 than in 1926. Almost identical counts were obtained in the 1926 and 1927 lots taken from the same sections as the Chicago test berries. The New Jersey berries in the holding tests also showed better keeping quality, in some cases, in 1927 than in 1926, but in the particular lot of Howes used in the storage experiment in Chicago there was slightly more rot in 1927 than in 1926.

Second—The prevailing temperature during shipping and at Chicago. As the berries were shipped and held under strictly commercial conditions, it was not possible to control the temperatures or even to record them exactly. The mean monthly temperatures for East Wareham and Boston, Mass., for Lakewood, N. J., and Chicago, Ill., show, however, that the temperature averaged from 4 to 5° F. higher during October and November of 1927 than in 1926. The approximate storage temperatures for the two years in Chicago are given in table 2.

That a difference in temperature of 4–5° F. is sufficient to account for the difference in amount of decay is indicated by the known effect of temperature on the fungi concerned and on the cranberries themselves. The careful studies of Morse and Jones[3] (p. 81) show that within the temperature ranges covered by this storage test the rate of respiration as measured by carbon dioxide given off approximately doubles with a rise in temperature of 10° C. (18° F.).

Fusicoccum putrefaciens,[4] which, when all the lots are considered, is the most destructive fungus in storage, is able to grow somewhat at 32° F. and is the only important rot fungus capable of appreciable growth below 50° F. While it grows more rapidly at temperatures above 50° F., its rate of

[3] MORSE, F. W., and C. P. JONES. Studies of cranberries during storage. Mass. Agr. Exp. Sta. Bul. 198. 1920.

[4] STEVENS, N. E. Temperatures of the cranberry regions of the United States in relation to the growth of certain fungi. Jour. Agr. Res. 11: 521–529. 1917.

TABLE 2.—*Approximate storage temperatures, Chicago, in degrees F.*

Month	1926	1927
October	65–55	70–60
November	53–43	58–48
December	40–33	48–35

acceleration is less than that of many other cranberry rot fungi. For example, its rate of growth increases less than two and one-half times from 50 to 68° F., while that of *Guignardia* increases three times and *Glomerella* six times with the same rise in temperature. During more than half the storage period of 1926 the temperature was too low for *Guignardia* and *Glomerella* to grow appreciably and these two fungi could grow but slowly earlier in the season. The cultures showed that these fungi caused a relatively small percentage of the rot in 1926. The 1927 storage temperatures were more favorable to *Guignardia* and *Glomerella*, and the cultures showed that in nearly every case the increase in these two fungi would largely account for the total increase in rot during 1927 over that in 1926.

Third—Further confirmation of the idea that the difference in storage temperatures adequately explains the different results for the two seasons is found in the fact that duplicate lots from Massachusetts stored at higher temperatures in Washington, D. C., showed a still greater amount of spoilage with corresponding increases in the percentage of the various fungi.

The writers must admit failure in their primary object, which was to secure an adequate basis for comparison of the fungi present in the cranberry crops of similar areas during successive years. The difference in shipping and storage temperatures have obscured any difference that may have existed at the time of harvest. The results of the test are of interest as showing exactly what causes of decay were active under actual commercial conditions in 1927 as compared with 1926.

SUMMARY

Cranberry storage tests with two varieties of berries from the four principal growing regions of the country were carried out in 1927 under the same commercial conditions as in 1926.

Compared with 1926, a greater amount of spoilage developed in 1927 in Massachusetts, New Jersey, and Oregon berries and a smaller amount in Wisconsin berries.

The increase in spoilage of the 1927 berries over that in 1926 was due chiefly to an increase in fungous rots, principally those that are most active at higher temperatures.

Holding tests on the bogs, on the contrary, indicated that both Massachusetts and New Jersey berries were actually sounder when picked in 1927 than in 1926.

Analysis of temperatures disclosed that the shipping and storage season for cranberries averaged from 4 to 5° F. warmer per day in 1927 than in 1926.

The increased amount of spoilage in 1927 is evidently attributable to the warmer season rather than to greater initial infection of the berries.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
AND
WISCONSIN DEPARTMENT OF AGRICULTURE.

NOTE ON THE OCCURRENCE OF INTRACELLULAR BODIES IN SPIKE DISEASE OF SANDAL (SANTALUM ALBUM LINN.)

M. J. NARASIMHAN

The spike disease of sandal has been known to occur in South India for the last 30 years, causing an annual loss of six lacs of rupees or about 150,000 dollars. The main symptoms of the disease are: reduction in the size of the leaves, shortening of the internodes, phyllody of the flowers, and death of the root ends and haustoria, leading to the death of the tree. The most striking feature is the accumulation of starch in the leaves and twigs.

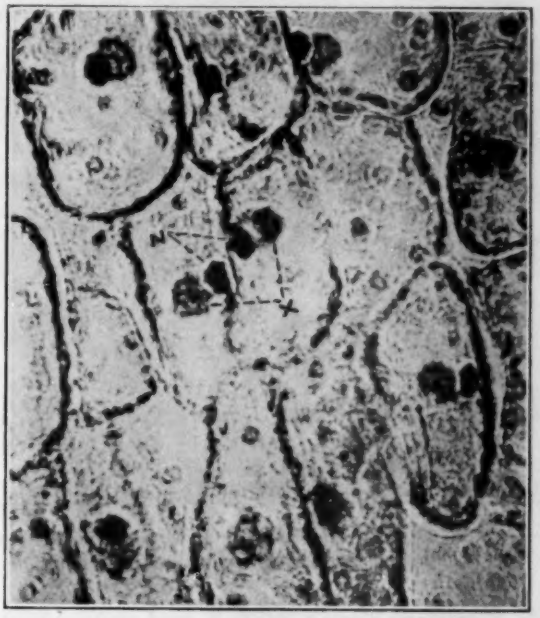


FIG. 1. Photomicrograph showing the bodies (X) associated with nuclei (N) in the cells of spiked leaf of sandal. ×1000.

There has been considerable literature dealing with the etiology of the spike disease of sandal, based on the assumption that unfavorable environmental conditions, such as forest fires, water-logging, drought, etc., and unsuitable host plants such as *Lantana*, played the main rôle in producing the disease, but Dr. L. C. Coleman[1] emphasized the fact that the appearance

[1] Coleman, L. C. Spike disease of sandal. Dept. Agr., Mysore, Mycol. Series, Bul. 3. 1918.

of the disease had nothing to do with the parasitic nature of sandal, and established the infectious nature of the disease by means of grafting experiments.

Cytological investigation of this disease, taken up by the author during 1927, revealed the presence of intracellular bodies in the cells of the spiked leaf. The bodies are round or oval-shaped, vacuolate, and are found in close association with the nuclei or occasionally free from them. Generally, only a single body is found in a cell, though there are cases where two to three of them occur (Figs. 1 and 2). The bodies are readily made out in

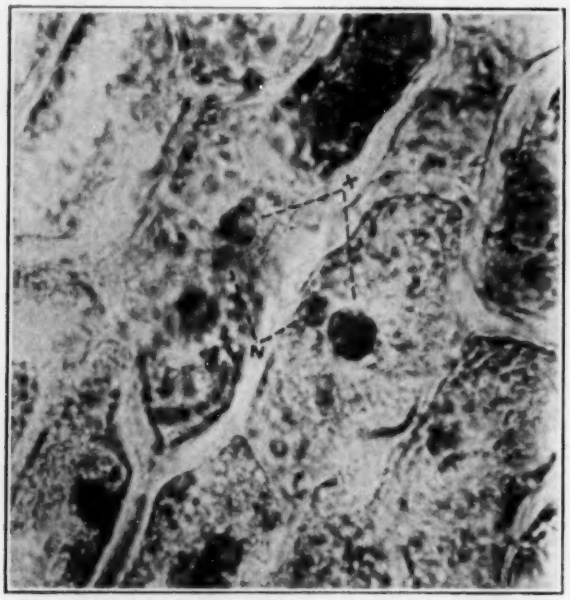


FIG. 2. Photomicrograph showing the bodies (X) free from the nuclei (N) in the spiked leaf of sandal. The vacuolated nature of the bodies can be seen. ×1000.

material fixed in Flemming's weak solution, and stained with Haidenhain's iron-alum haematoxylin or Flemming's triple stain. In the latter case, the bodies take up the Orange G. Indications of the degeneration of the nuclei in some of the affected cells are not wanting. The bodies have not been found in healthy leaves so far examined.

Similar bodies (Fig. 3) have been found by the author in the leaves of *Vinca rosea* Linn. affected by a similar disease. The finding of these bodies in spiked sandal and *Vinca* indicates the similarity of the disease in the two cases, and militates against the view held by some of the workers that the spike disease in sandal is caused by its association with unsuitable host

plants. The occurrence of bodies in spiked sandal similar to those found by various workers in a number of virus diseases of plants, such as the mosaic diseases of tobacco and *Hippeastrum*, rosette disease of wheat, etc.,

FIG. 3. Photomicrograph showing the bodies (X) in spiked leaf of *Vinca rosea*, some attached to the nucleus (N) and some free from it. ×500.

and in virus diseases of animals, lends support to the virus theory of the spike disease, put forward by Dr. Coleman.

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